GONADOTROPIN PULSATILITY IN FEMALE LONG DISTANCE RUNNERS

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YU-YAHIRO

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ABSTRACT

Title of Dissertation: Gonadotropin Pulsatility in Female Long Distance Runners

Janet Allison Yu-Yahiro, Doctor of Philosophy, 1986

Dissertation Directed by: Dr. Prabir K. Chakraborty, Ph.D.
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With the increased popularity of endurance athletics among women, exercise-associated menstrual irregularities have become more common. The object of this study was to determine whether eumenorrheic (EU) runners demonstrate any degree of abnormality in pulsatile gonadotropin release when compared to EU sedentary controls and to identify changes that could signal disruption of normal menstrual cyclicity. Gonadotropin pulsatility was measured by taking venous samples every fifteen minutes for six hours in age and weight matched endurance athletes (N=10) and sedentary controls (N=9) during early follicular phase. Athletes performed a one hour treadmill run at 75% of maximum oxygen consumption immediately followed by a second six hour gonadotropin pulsatility assessment. Data collected on all subjects included a maximum EKG treadmill test, body fat analysis, and determination of serum progesterone (P), estradiol (E2), testosterone (T), cortisol (C), and Beta-endorphin (B-end) concentrations. The frequency of the LH pulses did not differ significantly between the two groups but the amplitude of the LH pulses and the mean resting serum LH levels were significantly ($p<0.05$) higher in runners vs. controls (amplitude:

3.50 ± 0.44 vs. 1.45 ± 0.58; mean level: 4.37 ± 1.01 vs. 1.46 ± 0.20, mIU/ml respectively). There were no significant differences in mean FSH level (7.08 ± 1.18 vs. 7.24 ± 0.47 mIU/ml), pulse amplitude (4.10 ± 0.78 vs 5.06 ± 1.32 mIU/ml), or pulse frequency (3.14 ± 0.59 vs 3.00 ± 0.62 mIU/ml) between runners and controls. Resting levels of serum E2 were significantly lower in runners than controls (68.0 ± 4.0 vs. 88.6 ± 13.2 pg/ml; $p<0.05$). Serum E2 in runners was significantly higher after a one hour training run when compared to pre-exercise levels (E2 88.0 ± 11.0 vs. 114.2 ± 16.2 pg/ml; $p<0.05$). There were no differences in P or T concentrations either between groups or before and after exercise but luteal phase P were clinically subnormal in runners. B-end levels increased significantly ($p<0.05$) in both groups after the maximum treadmill test (runners: 2.16 ± 1.12 vs. 15.50 ± 3.76; controls 0.59 ± 0.10 vs. 9.42 ± 4.40 fm/ml; pre vs. post exercise), but there was no change in B-end levels in runners after a one hour training run. There were no differences in serum C levels between groups at rest or as a result of maximal or submaximal exercise. These data suggest that the acute increases in E2 levels accompanying daily training runs, combined with chronically depressed serum E2 levels, may result in a disruption of the normal steroid feedback control of gonadotropin release. Over time, this functional impairment of the feedback system may lead to the development of amenorrhea or oligomenorrhea.

GONADOTROPIN PULSATILITY
IN FEMALE
LONG DISTANCE RUNNERS

by

Janet Allison Yu-Yahiro

Thesis submitted to the Faculty of the Department of Physiology Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy 1986

DEDICATION

This dissertation is dedicated in loving memory of my father whose love for his family and dedication to his profession were truly worthy of admiration.

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I would first like to thank my advisor, Dr. Prabir K. Chakraborty, not only for his guidance through my thesis project and graduate education, but also for his concern for my intellectual and personal development as a research scientist. Many thanks are extended to my committee members, Dr. David Cruess, Dr. Jack McKenzie, Dr. Gregory Mueller, Dr. James Terris, and Dr. Robert Vigersky for their assistance and advise in all aspects of my thesis project and graduate career. The technical assistance of Miss Jeannie DeBolt, Dr. Alan Glass, Mrs. Susan Kyle, Dr. Gerard Letterie, Miss Leslie Montgomery, and Dr. Eric Schoomaker in performing the treadmill tests was invaluable and greatly appreciated.

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Lastly, I would like to thank my husband, Martin, for his continual love, unselfishness, and patience throughout this project and my entire graduate career. He was my tutor, my editor, and my biggest source of support and without him, completion of this project would not have been possible.

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I. INTRODUCTION

Within the past two decades women's distance running has made tremendous strides. Dr. Ernest Van Aaken organized the first all women's marathon in 1972. There were 45 entrants and 40 finishers. In contrast, the 1982 Avon Women's Marathon had over 5,000 participants. As recently as 1967 the longest Olympic event for women was the 800 meter run. The Olympic Committee believed that the "weaker sex" could not run further than a half of a mile. At that time the Boston Marathon was an all male event and when Katherine Switzer attempted to run disguised as a male, she was physically pushed off of the course. In 1982 the first Women's Olympic Marathon was run and won in a time of two hours and twenty-eight minutes, a vast improvement over the 1968 record of three hours and fifteen minutes.

However, with the genesis of the female distance runner and the continual increase in the caliber of her performance, the effect of long distance running on the menstrual cycle is now being questioned. In the late 1970's both coaches and physicians reported individual incidents of menstrual disorders in runners who trained at high intensities on a regular basis. The incidence of menstrual irregularities among athletes has been reported to be from 9% (Zaharieva, 1965) to as high as 20% (Feicht, 1978). Today, a number of

controlled studies have documented that secondary amenorrhea and oligomenorrhea are common in female athletes such as long distance runners and ballet dancers. To date, however, the body of research existing on this topic has been unable to conclusively establish a mechanism for these exercise-associated menstrual irregularities.

Researchers began to study exercise-induced amenorrhea because of the possible ramifications on the female athlete's fertility. While some physicians worried that exercise would produce permanent infertility, others hailed running as a new form of birth control. Exercise-associated menstrual irregularities now appear to be reversible with a decrease in physical activity but amenorrhea has recently been linked to another problem. Young female athletes may be in danger of developing early osteoporosis. After Cann (1982) observed a high incidence of stress fractures among his patients who were amenorrheic long distance runners, he launched a study and found significant premature bone loss in nonmenstruating sportswomen. Later, Drinkwater (1984) also found decreased vertebral bone mass in amenorrheic as compared to eumenorrheic athletes.

Etiologic factors which have been suggested as having significance in these disorders include a decrease in percent body fat (Schwartz, 1981), an "energy drain" caused by increased energy output (Warren, 1980), augmented prolactin release following exercise (Brisson, 1980), hyperandrogenism (Cumming, 1983) and the excess psychological stress of competition (Schwartz, 1981). In addition, exercise-

associated changes in blood minerals (Kyle, 1986), increased central nervous system B-endorphin activity (McArthur, 1980), and exercise-induced rises in catecholestrogen levels (Russell, 1984a) are believed by some to contribute to the onset of these menstrual irregularities. The differences in conclusions among these studies may reflect, in part, differences in subject selection and often the lack of an appropriately matched control group.

Most studies have revealed that the amenorrheic athlete is hypoestrogenemic and has an older than average age at menarche (Erdelyi, 1962). A recent study by Veldhuis (1985a) has shown a decreased gonadotropin hormone releasing hormone (GnRH) pulse frequency in amenorrheic runners. Also Ronkainen 1985b found that the mean luteinizing hormone (LH) and follicle stimulating hormone (FSH) responses to GnRH were significantly lower in eumenorrheic runners compared to controls. These studies are interesting in light of increasing evidence that the endogenous opiates increase with exercise and that they may inhibit gonadotropin release from the anterior pituitary through control of the activity of GnRH neurons in the hypothalamus. For instance, running has been demonstrated to elevate plasma B-endorphin levels as much as sixfold (Bortz, 1981).

Generally, studies have compared amenorrheic athletes to either eumenorrheic athletes or sedentary controls. While these studies provide a valuable description of the amenorrheic athlete, their failure to uncover the mechanism

for the development menstrual disturbances may be due to the fact that the disruption, probably somewhere in the hypothalamic-pituitary-ovarian axis, happened in the past and adaptations have most undoubtedly occured. This study endeavored to uncover differences in reproductive hormone levels and specifically in gonadotropin pulsatility and serum B-endorphin levels, between elite eumenorrheic distance runners and age-matched sedentary controls. It was thought that if differences in reproductive hormone patterns were found to exist between these two groups, it might lead to a better understanding of the process by which amenorrhea is developed.

The following review will discuss the normal menstrual cycle, gonadotropin pulsatility and factors influencing gonadotropin release, and the role of the endogenous opiates in the control of the menstrual cycle. Studies concerning the effect of exercise on reproductive steroids, gonadotropin pulsatility and B-endorphin release will be compared and contrasted. In addition, exercise-induced amenorrhea and the menstrual cycle in eumenorrheic athletes will be discussed.

II. REVIEW OF LITERATURE

A. Normal Menstrual Cycle

The typical north American woman attains menarche between ages nine and sixteen years at which time she begins to menstruate and continues to do so until menopause unless intervened by pregnancy. The normal menstrual cycle ranges from 25 to 30 days in length. It can be divided into the follicular or proliferative phase, ovulation, and the luteal or secretory phase as illustrated in figure 1.

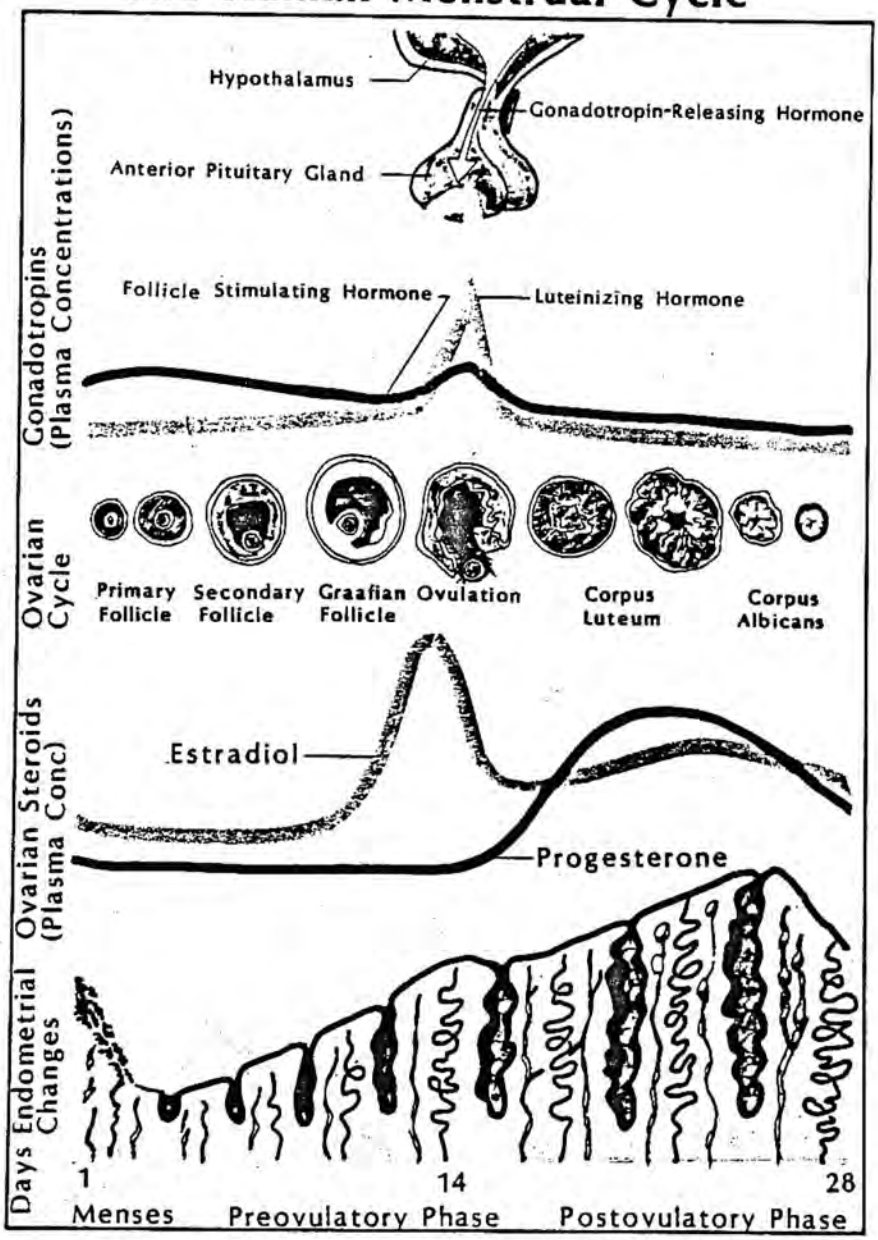
1. Steroid and Gonadotropin Levels During the Menstrual Cycle

During the early follicular phase, the level of estradiol is low, generally less than 50 to 100 picograms per milliliter (pg/ml). About one week prior to the preovulatory luteinizing hormone (LH) surge, estradiol begins to rise reaching a peak of 200 to 300 pg/ml approximately one day before the LH surge. The estradiol level then drops precipitously prior to ovulation. There is a second rise in plasma estradiol levels during the mid luteal phase. While estradiol comes primarily from ovarian granulosa cells during the follicular phase, the mid luteal estradiol rise is due to its production by the corpus luteum.

Plasma progesterone concentrations are low, less than one nanogram per milliliter (ng/ml) during the early

Figure 1 An illustration of the human menstrual cycle showing the changes in gonadotropin and ovarian steroids throughout the cycle. The development of the ovarian follicle and endometrium are also shown.

The Human Menstrual Cycle



follicular phase, and this progesterone is from both adrenal and ovarian sources. Progesterone levels begin to increase during the LH surge, reaching a plateau of 10 to 20 ng/ml, and remain elevated throughout most of the luteal phase. This progesterone is derived from the preovulatory ovarian follicle and later the corpus luteum. During the latter third of the luteal phase, progesterone levels decline rapidly.

In normal cyclic women, small quantities of testosterone are secreted by both the ovaries and adrenal glands. More than half of this circulating hormone arises from peripheral conversion of androstenedione to testosterone. The normal plasma concentrations of testosterone range from 0.2 ng/ml to 0.4 ng/ml. There is little change in plasma testosterone levels during the menstrual cycle except for a small increase during the preovulatory phase due to a midcycle androstenedione surge.

The gonadotropins, luteinizing (LH) and follicle stimulating hormone (FSH) are low during the early follicular phase (LH: 5-25; FSH 5-20 mIU/ml). They rise gradually during the mid follicular phase and then surge simultaneously for about 36 hours before ovulation (LH: 25-100; FSH 12-30 mIU/ml). During the luteal phase they are once again low (Wilson, 1985).

2. Control of Growth and Steroidogenesis in Ovarian Follicles

The menstrual cycle begins with the development of primordial follicles. Follicle stimulating hormone binds to

primordial follicles. Follicle stimulating hormone binds to FSH receptors on the granulosa cells of the follicles causing the induction of LH receptors, estrogen production, and of enzymes with aromatase activity for the conversion of androgens to estrogens. The estradiol produced acts locally to cause proliferation of the granulosa cells and an overall increase in the size of the follicles. Estradiol also has a permissive action on the induction of LH receptors by FSH. Luteinizing hormone in turn stimulates the synthesis of androgen precursors.

During the late follicular phase, coincident with the rise in estradiol, the preovulatory follicle begins a phase of rapid growth. It is believed that estradiol stimulates an increase in the number of granulosa cells and in the volume of antral fluid as well as a hypertrophy of the thecal cells. At the time of ovulation, usually a single follicle is selected while the other maturing follicles undergo atresia. The mechanism by which this selection occurs is not understood but may be related to the antral fluid hormone milieu which differs from follicle to follicle (Wilson, 1985).

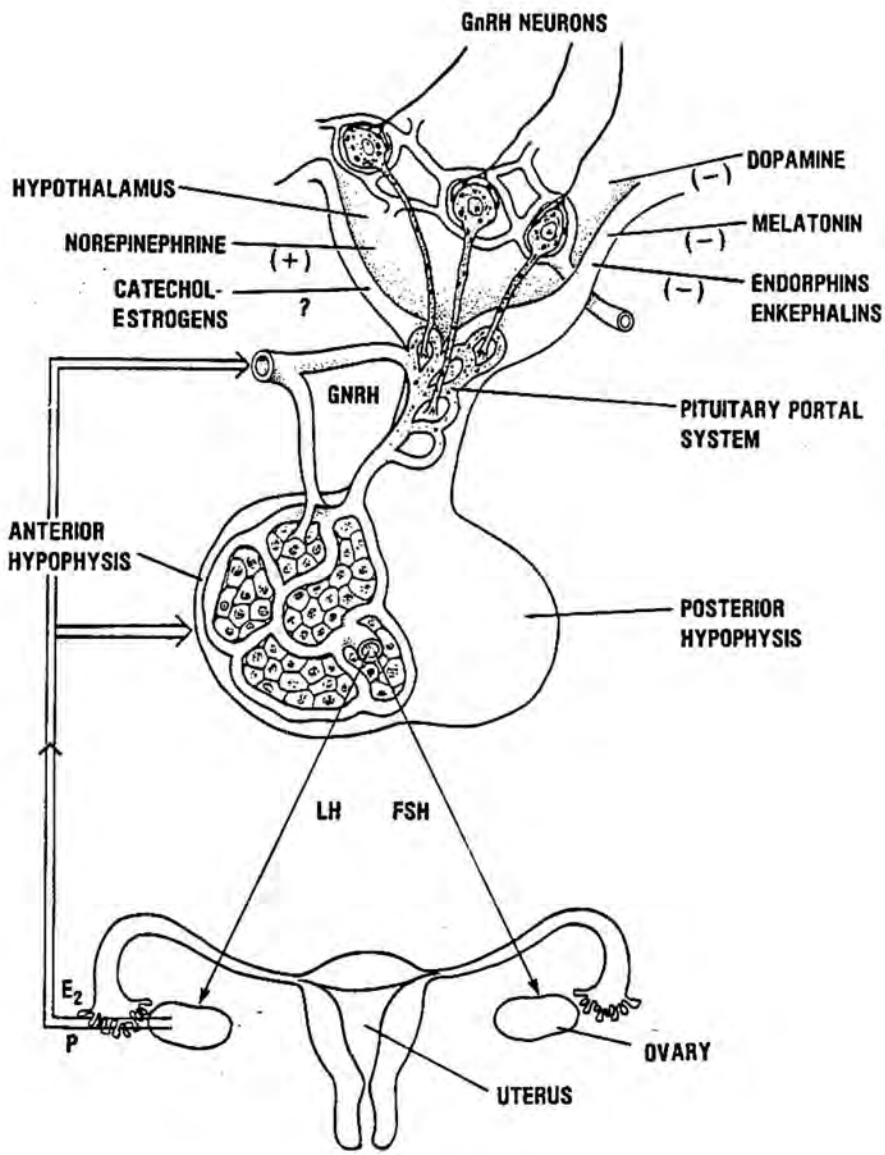
The LH surge stimulates ovulation causing the follicle to burst and the ovum to be released. The empty follicle is then transformed into the corpus luteum. Luteinization of remaining granulosa cells is stimulated by LH or human chorionic gonadotropin (hCG) through a mechanism involving stimulated cAMP production and progesterone secretion by the

corpus luteum. During luteinization, plasma estradiol levels fall then rise gradually. The presence of luteinized theca cells is thought to be necessary for the corpus luteum to produce estradiol. Estradiol may also have a luteolytic function in humans. About fourteen days after ovulation the corpus luteum undergoes regression, stops steroid production, and estradiol and progesterone levels drop dramatically.

3. Development of the Endometrium

During menstruation, bleeding occurs due to a sloughing off of the endometrium from the previous menstrual cycle. The endometrium begins to grow again during the early follicular phase. There is a proliferation of the epithelial cells of the uterine mucosa and the surface layer begins to fold into crypts. The increased estradiol levels in the preovulatory phase causes a thickening of the mucosa, a lengthening of the crypts, and an elongation of the spiral arteries. During the luteal phase progesterone induces a coiling of the crypts and an increased vascularization of the endometrium. At the end of the luteal phase, coincident with the declining steriod levels, the spiral arteries contract, possibly due to a prostaglandin effect. The endometrium, then lacking a sufficient blood supply, becomes necrotic and is sloughed off initiating the next menstrual cycle.

Figure 2 A schematic diagram illustrating the hypothalamic-pituitary-ovarian axis. Also shown are some possible regulators of GnRH release.


GnRH NEURONS
HYPOTHALAMUS
NOREPINEPHRINE
(+)
CATECHOL-
ESTROGENS
?
DOPAMINE
(-)
MELATONIN
(-)
ENDORPHINS
ENKEPHALINS
(-)
GNRH
PITUITARY PORTAL
SYSTEM
ANTERIOR
HYPOPHYSIS
POSTERIOR
HYPOPHYSIS
LH
FSH
E_2
P
OVARY
UTERUS

B. Gonadotropin Regulation During the Menstrual Cycle

1. Overview of Gonadotropin Release

Gonadotropin releasing hormone (GnRH) is a decapeptide synthesized and released in the hypothalamus, particularly in the arcuate and paraventricular nuclei. This releasing hormone travels down through the portal system and binds to receptors on the gonadotrophs of the anterior pituitary stimulating the release of the gonadotropins, LH and FSH. The gonadotropins then travel through the blood stream to the ovaries causing the release of estradiol and progesterone, estradiol primarily from the ovarian follicles and progesterone mainly from the corpus luteum (figure 2).

Both LH and FSH are localized within the pituitary basophilic gonadotroph cell. Luteinizing hormone and FSH are glycoproteins and are composed of an alpha and beta polypeptide chain. The alpha chain for LH, FSH, thyroid stimulating hormone and human chorionic gonadotropin is identical and is composed of 89 amino acids. Although it is the beta chain that confers to each hormone its biologic specificity, both chains are essential for full biological activity. The beta subunits for LH and FSH are 115 amino acids long and similar except for a difference in 30 amino acids (Greenspan, 1983).

Recent studies indicate that there are two pools of gonadotropins, a readily releasable pool, and a storage pool (Ferin, 1984a). The first is sensitive to small changes in GnRH while the second requires prolonged GnRH stimulation.

The pituitary capacity is the total activity of both pools and this capacity changes throughout the menstrual cycle.

Pituitary capacity is low during the early follicular phase and begins to rise during the midfollicular period. It remains high throughout the early luteal phase and then has a mid-luteal decline. During the midfollicular phase there is a greater rise in the storage pool than the readily releasable one. However, near ovulation the reverse occurs, the rise in the readily releasable pool exceeding that of the storage pool (Yen, 1980).

2. Steroid Influence on Gonadotropin Release

The release of gonadotropins from the pituitary is mediated by GnRH and modulated by the ovarian sex steroids. Gonadotropin releasing hormone has two roles: at high concentrations GnRH stimulates the release of gonadotropins while at low levels it has a priming role, sensitizing the gonadotrope for subsequent GnRH mediated gonadotropin release. In addition, both GnRH and estradiol promote gonadotropin synthesis. However, estradiol also has an inhibitory effect on GnRH stimulated gonadotropin release and at high concentrations, estradiol augments the priming role of GnRH (Yen, 1976).

Estradiol has both negative and positive feedback effects on the release of gonadotropins. It acts at the pituitary, as previously described, as well as at the hypothalamus to modulate the frequency and amplitude of GnRH pulses. During the early follicular and luteal phases, the

pituitary is the primary site of the classic negative feedback action of estradiol on gonadotropin release (Pohl, 1982). Estradiol exerts a positive feedback effect on gonadotropin release just prior to ovulation.

First, estradiol levels begin to rise during the midfollicular phase, stimulating an increase in the gonadotropin storage pool. Then about 72 hours before ovulation, estradiol reaches a critical level, approximately 300 pg/ml. In combination with the priming and releasing actions of GnRH, this high level of estradiol acts by a positive feedback mechanism to stimulate the midcycle LH surge. Progesterone also rises slightly at ovulation synergising the positive feedback action of estradiol on gonadotropin release. In addition there may also be some as yet unidentified triggering mechanisms involved.

Recent evidence indicates that high progesterone levels during the luteal phase may be responsible for the increased amplitude and decreased frequency of LH pulses observed (Soules, 1984; Brody, 1984). In these studies normal gonadotropin pulsatility was measured during the follicular phase. Subjects then received progesterone either intramuscularly for six days (Soules) or intravenously for 24 hours (Brody). In both cases the frequency of the LH pulses decreased and the amplitude increased significantly after progesterone administration so that they strongly resembled luteal phase pulsatility patterns. In fact, when these were compared to the normal mid-luteal pulse patterns of these

subjects, they were not significantly different. These results suggest that progesterone acts centrally to modulate pulse frequency in the hypothalamus. Merriam (1984a) suggests that progesterone also acts at the pituitary level to increase LH pulse amplitude during the luteal phase. In addition, progesterone is also believed to have a role in timing or induction of the pre-ovulatory LH surge since approximately 16 hours prior to the mid-cycle LH peak, progesterone levels begin to rise progressively (Hoff, 1983; Collins, 1984).

3. Normal Gonadotropin Pulsatility

Luteinizing hormone pulse frequency changes throughout the menstrual cycle. During the follicular phase there are low amplitude, high frequency pulses while in the luteal phase the pulses are of high amplitude and low frequency. There is no agreement on the specific number of pulses during a single phase of a normal menstrual cycle or on the causes of changes in gonadotropin pulsatility throughout the cycle. Results of pulsatility studies performed on normally menstruating women vary greatly due to differences in the phase of the cycle during which a study was conducted as well as to differences in sampling frequency and methods of pulse analysis.

Rossier (1977) and Yen (1972) found one LH pulse every 90 minutes in the early follicular phase and one pulse every 1-4 hours throughout the luteal phase. Yen also found that the LH pulse amplitude during the luteal phase was two times

greater than during the follicular phase (10-30 mIU/ml vs. 5-15 mIU/ml). Santen and Bardin (1973) found an even greater difference in LH pulse amplitude, 73±15 ng/ml in the mid-luteal vs. 26±5ng/ml in the mid-follicular. This group also found fewer pulses in the mid-follicular phase than Yen, 3.2±0.36 pulses in 16 hours, while the frequency of the luteal phase pulses was similar.

Backstrom (1982) first showed that the frequency of LH pulses increases significantly from the early to the late follicular phase of the menstrual cycle (3.4±0.3 vs. 4.4±0.2 pulses per six hours). However, in contrast to Yen (1972) and to Santen and Bardin (1973) who found high frequency, low amplitude pulses during the follicular phase and low frequency, high amplitude pulses in the luteal phase, Backstrom (1982) found that the LH pulse amplitude and mean LH value were lower in the luteal than the follicular phase (amplitude 7.4±1.7U/L vs. 25±1.0 U/L and mean LH 5.6±0.9 U/L vs. 10.0±2.0 U/L; luteal vs. follicular phase). Estradiol pulses were also detected in this study with 74 to 80% of all LH pulses followed within 100 minutes by an estradiol pulse. During the early follicular phase the estradiol pulse frequency was similar to that of LH although estradiol pulse frequency did not increase from the early to the late follicular phase.

Reame (1984) also found an increase in LH pulse frequency from the early to the late follicular phase of the menstrual cycle. This study was unique in that it studied

the same women at seven day intervals during a single menstrual cycle. Samples were drawn at ten or twenty minute intervals for 12 or 24 hours and pulses were analysed by the author's method, a modification of the Santen and Bardin (1973) technique, or by the Cycle Detector Program of Clifton and Steiner (1984). Reame found a higher LH pulse frequency using the ten minute sampling frequency for all phases of the menstrual cycle. In a 12 hour period she found 11.8±Ø.6 pulses during the early follicular, 14.3±1.Ø pulses during the late follicular, 8.Ø±2.Ø pulses in the mid-luteal and 7.8±1.Ø pulses in the late luteal phase. This study also found that the LH pulse amplitude varied little throughout the follicular phase, rose during the mid-luteal phase then fell toward the end of the cycle.

Filicori and Crowley (1983) found approximately one pulse per hour during the follicular phase when they sampled every ten minutes for 24 hours. These results are in agreement with those of Brody (1984) who analysed pulsatility utilizing the PULSAR program. In both the Reame (1984) and Filcori (1983) studies, secretory patterns were highly variable during the luteal phase, perhaps due to the prolonged exposure to high levels of estradiol and progesterone. Like Backstrom (1982), Reame (1984) also found that 75 to 8Ø% of the LH pulses were followed by E2 secretion during the follicular phase.

It is difficult to detect FSH pulses since smaller amounts of FSH are released in response to a GnRH pulse. In addition the half life of FSH is 3.9 hours, much longer than

the 40 minute half life of LH so that an FSH pulse may be masked by the slow decay of the previous pulse. Most studies have found FSH pulses in some but not all of their subjects and have not found a correspondence of LH and FSH pulses (Reame, 1984; Sander, 1984). In contrast, Soules (1984) found that FSH levels had a positive correlation with LH pulse activity in the three phases of the menstrual cycle during which he tested: early follicular, late follicular, and mid-luteal. He states, however, that classical pulse analysis of FSH was not possible since FSH pulse amplitude was so low.

Yen (1972) saw no FSH pulses in any of his pre-menopausal subjects during an eight hour period. Backstrom (1982) found that as with LH there was an increase in FSH pulse frequency from the early to late follicular phase of 2.3 ± 0.3 and 3.88 ± 0.3 pulses per six hours, respectively. In general, FSH is much less variable than LH throughout the cycle. Backstrom (1982) attributed this to ovarian influences such as estradiol which have a differential effect on the responsiveness of the anterior pituitary to GnRH with respect to LH and FSH.

Recently, Veldhuis and colleagues (1986) performed an exhaustive study in an effort to demonstrate a stable estimate of the physiological LH pulse frequency. These authors believe that former experiments with "arbitrary sampling protocols" for estimating gonadotropin pulsatility have been in error by as much as fivefold. Eight normal women in the early follicular phase of their cycles were

sampled every five minutes for 24 hours. Using a modification of the Santen and Bardin (1973) method for pulse analysis they found an average of 20.6±3.6 pulses per 24 hours. Their results showed that more pulses were detected with a greater sampling frequency and a longer sampling duration. From these results it was estimated that only 50 percent of the pulses actually occurring would be detected if sampling was done every 18.9 minutes. Sampling at 2.0 minute intervals would be required to detect 90 percent of all of the pulses. The results of the studies cited above on gonadotropin pulsatility during a normal menstrual cycle are summarized in table 1.

4. Other Factors That Influence Gonadotropin Release

The current understanding of the mechanism by which GnRH is released from its neuron is complicated and controversial. A variety of neurotransmitters and hormones may be involved including dopamine, norepinephrine, estrogen, progesterone, the endogenous opiates, and the prostaglandins. In addition, catecholestrogens may play a role in pathological and possibly under normal conditions (figure 2).

One hypothesis for the regulation of GnRH is that it is modulated by a dual catecholaminergic system whereby norepinephrine stimulates and dopamine suppresses GnRH release (Yen, 1980). The arcuate-median eminence region of the hypothalamus has dopamine and norepinephrine containing neuronal pathways that impinge on GnRH neurons in this area.

Table 1 Comparison of LH pulse frequency and pulse amplitude found in current studies at various phases of the menstrual cycle.

TABLE 1-A

LH VALUES FOUND IN RECENT STUDIES

FOLLICULAR PHASE

Author	Collection Frequency	Early		Mid		Late	
		Pulse Freq.	Pulse Amp.	Pulse Freq.	Pulse Amp.	Pulse Freq.	Pulse Amp.
Yen, 1972	4 hrs	2	5-15mIU/ml	-	-	1	-
Santen, 1982	6 hrs	-	-	3.2±0.36	26±5 ng/ml	-	-
Backstrom, 1982	6 hrs	12.8±0.3	25±1 U/L	4.1±0.3	10.0±2.0 U/L	4.4±0.2	1.6±0.2 U/L
Brody, 1982	24 hrs	8.0±1.4	6.5±0.6 mIU/ml	12.2±1.0	5.2±0.2 mIU/ml	12.8±1.9	4.7±1.2 mIU/ml
Reame, 1984	12 hrs						
20 min interval		7.7±0.6	3.5±0.4 mIU/ml	-	-	7.4±0.9	2.9±0.4 mIU/ml
10 min interval		11.8±0.6	4.0±0.7 mIU/ml	-	-	14.3±1.0	3.3±0.6 mIU/ml
Soules, 1984	24 hrs	10.2±1.8	15.0±2.4 ng/ml	-	-	12.7±2.2	17.0±0.9 mIU/ml
Veldhuis, 1985	24 hrs	10.6±0.3	31±18 mIU/ml	-	-	-	-
Veldhuis, 1986	24 hrs	20.6±3.6	-	-	-	-	-

Table 1-B Veldhuis (#7 and #8) did not sample women during the late follicular or luteal phases in either of his studies.

TABLE 1-B

LH VALUES FOUND IN RECENT STUDIES

LUTEAL PHASE

Author	Collection Frequency	Mid		Late	
		Pulse Freq.	Pulse Amp.	Pulse Freq.	Pulse Amp.
Yen, 1982	4 hrs.	1			
Santen, 1982	6 hrs.	1.6±0.3	73±15 ng/ml	-	-
Backstrom, 1982	6 hrs.	7.4±1.7	-	-	-
Brody, 1982	24 hrs.	10.5±2.1	7.4±1.3 mIU/ml	4.0±0.6	8.0±2.9 mIU/ml
Reame, 1984	12 hrs.				
20 min interval		5.6±0.6	6.8±2.1 mIU/ml	5.3±0.0	7.8±1.0 mIU/ml
10 min interval		8.6±2.0	10.3±4.4 mIU/ml	4.7±1.3	6.3±2.4 mIU/ml
Soules, 1984	24 hrs.	4.5±0.9	24.7±66.0 ng/ml	-	-

Aside from their morphologic proximity, further evidence that there is an interaction among dopamine, norepinephrine, and GnRH is the finding that the LH surge is accompanied by changes in the activities of these biogenic amines (Yen, 1980). Yen (1976) demonstrated in the rat model that at the time of proestrous, there is a decrease in dopamine turnover and an increase in norepenephrine turnover in the median eminance.

When norepinephrine was applied to the rat hypothalamus, it evoked the release of LH from the pituitary (Rosner, 1976). An in vitro study showed that norepinephrine also stimulates the release of GnRH from median eminance slices (Negro-Villar, 1979). Rameriz (1986) demonstrated that norepinephrine is a potent stimulator of LHRH release in conscious unrestrained rabbits who had indwelling hypothalamic cannulae. In contrast, Condon (1986) found that intracerebroventricular infusion of norepinephrine in ovariectomized rats caused a significant inhibition of LH and of FSH to a lesser extent. Clonidine, an alpha-2 agonist, also caused a decrease in plasma LH but no change in FSH, suggesting independent regulation of LH and FSH.

Contrary to this model of dual catecholaminergic control, Negro-Villar (1979) showed that dopamine was as effective as norepinephrine in evoking LHRH release. However, Yen (1980) has shown that dopamine inhibition is most effective when gonadotropin levels are elevated. When gonadotropin levels were lowered in ovariectomized women by estrogen pretreatment, dopamine was much less effective in

suppressing LH levels than prior to treatment. The data of Kletzky (1986) are not in agreement with those of Yen (1980). Kletsky (1986) administered two dopamine agonists to ovulatory women throughout their cycles and found that dopamine inhibition of LH occured only during the follicular phase, when steroid hormones are lowest.

Leadman (1985) administered B-endorphin, naloxone, epinephrine, norepinephrine or prostaglandin-E2 intraventricularly to female rats. As in other studies, he found that B-endorphin blocked ovulation and that naloxone reversed this blockade. Additionally, epinephrine and norepinephrine were unable to induce ovulation in B-endorphin pretreated rats but prostaglandin-E2 stimulated LH release and ovulation in these rats. A second dose of epinephrine caused an LH rise but not sufficient to stimulate ovulation.

From these data, Leadman (1985) and collegues postulated a model for GnRH release. They believe that adrenergic transmitters bind to alpha adrenergic receptors on the GnRH neurons causing the release of prostaglandin-E2. Prostaglandin E2 in turn stimulates the release of GnRH from its nerve terminals. B-endorphin acts presynaptically by suppressing adrenergic activity in the preoptic-tuberal pathway. These findings indicate that "B-endorphin does not impair the ability of GnRH neurons to respond to excitatory signals acting at steps subsequent to adrenergic release". The authors also suggest that ovarian steroids may enhance adrenergic tone in the hypothalamus so that high mid-cycle

steroids would facilitate the preovulatory LH surge.

The findings of Miller (1985) do not agree with the Leadman model. She hypothesizes that if endogenous opiates act through a catecholamine intermediary to inhibit LH secretion, then rats with ascending norepinephrine tract lesions should not respond to naloxone with an increase in LH levels. However, when she gave naloxone to sham operated and norepinephrine tract-lesioned rats, both groups had a three to fourfold rise in LH levels. Therefore, from these findings as well as her current knowledge of this area, she developed her own model of GnRH release. She believe that norepinephrine has a tonic facilitatory effect on GnRH. An acute increase in norepinephrine hypopolarizes the GnRH neuron toward threshold potential. Opiates act, perhaps postsynaptically, to hyperpolarize the GnRH neuron. Naloxone would remove this hyperpolarization, effectively depolarizing the neuron above thresold potential so it could fire and cause GnRH release.

Catecholestrogens are metabolites of estrogen that contain a hydroxy group in either position two or four. This compound has both catecholamine and estrogen-like activities. Two-hydroxyestrogens are substrates for catechol-o-methyl-transferase (COMT) and have a higher affinity for COMT than do the catecholamines (Yen, 1980). Thus, through competition, they decrease the inactivation of catecholamines thereby increasing their effective concentrations. Parvizi (1980a) showed that 2-hydroxyestradiol-17beta injected into the amygdala of castrated miniature pigs inhibits LH

secretion within two hours of injection. Whether this is a direct inhibition or perhaps due to suppression of LH by increased levels of dopamine is uncertain. In addition, catecholestrogens bind with a high affinity to estrogen receptors in rat uterus (Davis, 1975). These results suggest that catecholestrogens may be potent endogenous antiestrogens. High concentrations of this compound could supress normal estrogen actions.

Russel (1984a) studied competitive swimmers over a two year span which encompassed periods of both moderate and heavy training. Interestingly he found that both catecholestrogens and B-endorphins were significantly elevated during the strenuous as compared to the moderate training period. Mean levels of LH FSH, prolactin, and estradiol were also significantly lower during the strenuous training period. These hormonal changes were accompanied by the development of oligomenorrhea in five of thirteen swimmers. During the moderate training periods normal menses resumed and hormone levels were no different than in an age-matched sedentary control group.

5. Measurement and Analysis of Gonadotropin Pulsatility

The secretion of luteinizing hormone and follicle stimulating hormone from the anterior pituitary is episodic rather than continuous. Ferin (1984) postulates that there is a "GnRH oscillator" or neural transducer in the medial basal hypothalamus which receives and integrates pertinant

endocrine and neural information and translates it into a signal of specific amplitude and frequency. Merriam (1984a) describes the episodic secretion by the GnRH neuron as being triggered by "bursting pacemaker membrane depolarizations". This pulsatile release of GnRH is important since a specific pulse frequency and amplitude are thought to be necessary for maintenance of normal menses.

Since it is not feasible in humans to measure GnRH levels in the hypothalamic-pituitary portal blood, its pulsatile patterns are measured indirectly by pituitary secretion of LH and FSH. However, in making these measurements it must be taken into account that the pituitary release of gonadotropins is affected by both central influences as well as the gonadal steroids as previously discussed.

There have been numerous methods employed to analyze gonadotropin pulsatility. Some of the earlier studies employed a manual technique where data was graphed and given to a scorer who visually identified all of the hormone peaks (Merriam, 1984b). Santen and Bardin (1973) were the first to measure episodic hormone secretion in a systematic fashion. They defined a pulse as "a rise of 20% or more from the immediately preceeding local minimum to the next local maximum" . The authors chose the criterion of a 20% rise because in their study this represented approximately three times their assay coefficient of variation. Others have modified Santen and Bardin's technique and define a peak as a certain multiple of the assay's standard deviation (Baird,

1978) or intraassay coefficient of variation above the mean (Moult, 1982).

Merriam and Wachter developed PULSAR in 1982. PULSAR begins by calculating "a smoothed baseline". This baseline differs from the mathematical mean, or baseline, in that it follows a hormone's diurnal variations and is insensitive to outliers. The baseline is then scaled in terms of either standard deviation or the intra-assay coefficient of variation, and cut-off criteria are established. In this program both a height and width criteria are employed. For luteinizing hormone there is an adjustable cut-off whereby the criteria for accepting a peak are more strict for narrower than for wider peaks.

Merriam and Wachter (1982) compared pulse analysis by PULSAR to that done visually, by the Santen and Bardin method, and by the HORMPART program in nineteen sets of LH data sampled every 20 minutes for 24 hours. In these data sets 118 LH peaks were identified visually. The Santen and Bardin program was more sensitive than the eye, it chose all 118 visually identified peaks plus 98 others. The HORMPART program only selected 51 total peaks, 44 of which were identified visually.

This study illustrates the methodological problems in analysing gonadotropin pulsatility as well as the difficulty in comparing the results of studies which employ different pulse analysis criteria.

Another confounding factor in doing pulse analysis is

the difference in the frequency of sampling. Veldhuis (1986) compared venous sampling rates of 20 minutes, 4 minutes, 2 minutes, and one minute to determine LH pulse frequency in menses. He found that the number of pulses determined by 4, 2, and 1 minute sampling frequencies revealed 3.6, 4.9, and 13.7 fold more LH pulses as compared to those measured at 20 minute intervals. The additional pulses unmasked were of high frequency and low amplitude. Merriam (1984b) addresses the problem of sampling frequency and cautions that higher sampling frequency increases the liklihood of false positive peaks, accepting an increase in LH as a peak when it is really assay artifact. He believes that a reasonable guideline would be to sample 2-3 times per half-life of the hormone or 5-6 times during the interval one expects to find between peaks, whichever is more frequent.

Luteinizing hormone has a half life of about 30 minutes and there is approximately one pulse every 90 minutes during the early follicular phase of the menstrual cycle. Therefore, following these guidelines, a sampling frequency of 15 minutes as chosen for this study, is appropriate. Since a 15 to 20 minute sampling interval was employed in most studies, which investigated gonadotropin pulsatility in eumenorrheic distance runners, selection of a 15 minute sampling interval will facilitate comparison of results among studies (Veldhuis, 1985a; Cumming, 1985a and b).

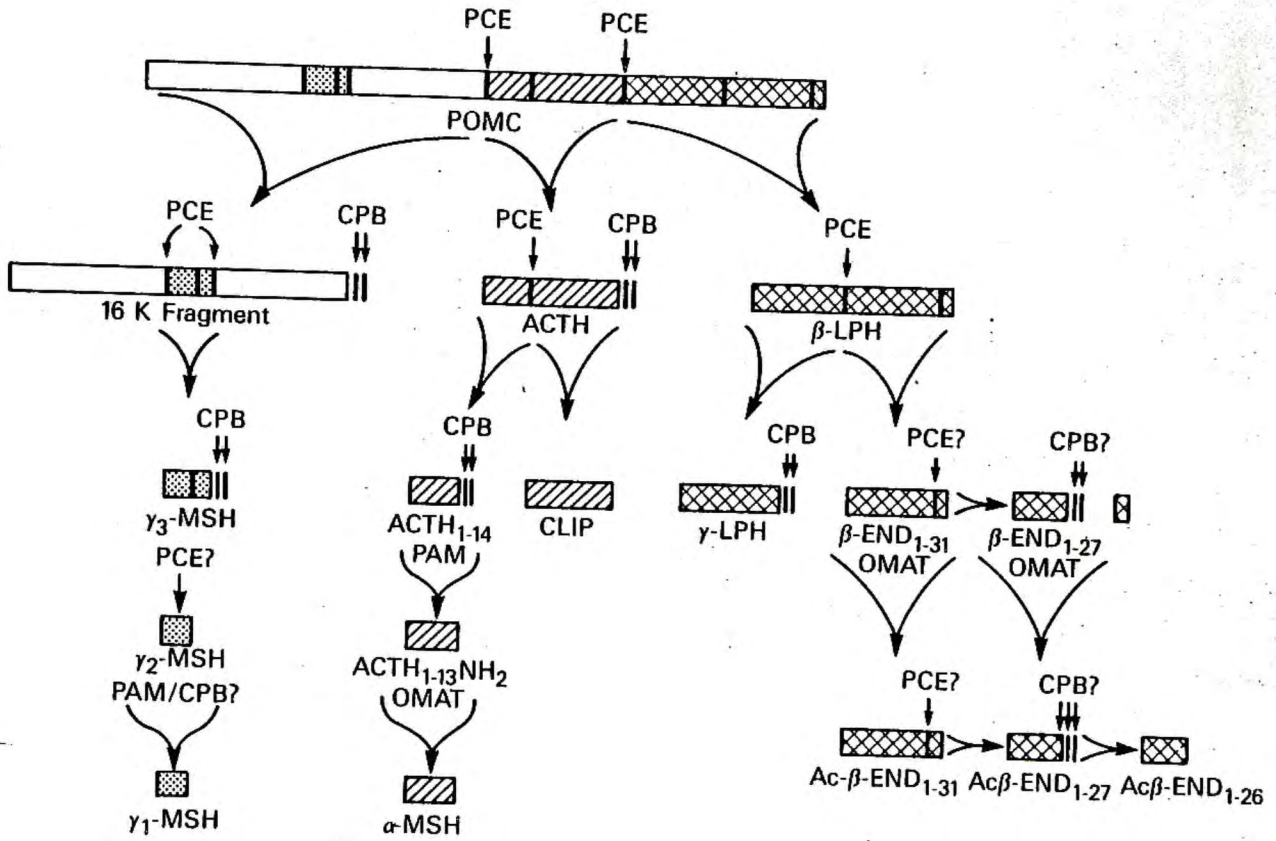
C. Beta-endorphin

1. General Description

According to Bloom, (1983), "The superfamily endorphin includes all endogenous peptides whose sequences include an enkephalin pentapeptide (either Met-enkephalin or Leu-enkephalin) and that share some common actions at presumptive opiate receptors as defined by naloxone antagonism...". Three families of endogenous opioid peptides have now been described: (1) those derived from proopiomelanocortin, including B-endorphin; (2) those originating from prodynorphin, dynorphins A and B and alpha-neoendorphin; and (3) the proenkephalin family from which Met- and Leu-enkephalin are derived (Faden, 1984). These substances may act as neurotransmitters, hormones, and neuromodulators and have been implicated in a wide variety of physiological and pathophysiological functions.

B-endorphin was first isolated by Li and Chung (1976) from camel pituitary glands. Shortly after this, B-endorphin was isolated from brain extracts independently by a number of researchers (Bradbury, 1976; Cox 1975; Loh 1976; Rubinstein, 1977). The pituitary gland contains the highest concentration of B-endorphin and is the primary source of circulating B-endorphin. The endorphin containing neurons in the central nervous system have a parallel distribution with opiate receptors. This centrally produced B-endorphin may be anatomically isolated from peripheral B-endorphin due to restricted transport across the blood brain barrier.

Figure 3 A schematic diagram illustrating the proteolytic processing of the 31K precursor, proopiomelanocortin.


PCE
PCE
POMC
PCE
CPB
16 K Fragment
PCE
CPB
ACTH
PCE
β-LPH
CPB
γ3-MSH
PCE?
γ2-MSH
PAM/CPB?
γ1-MSH
CPB
ACTH1-14
PAM
ACTH1-13NH2
OMAT
α-MSH
CLIP
CPB
γ-LPH
PCE?
β-END1-31
OMAT
CPB?
β-END1-27
OMAT
PCE?
Ac-β-END1-31
CPB?
Acβ-END1-27
Acβ-END1-26

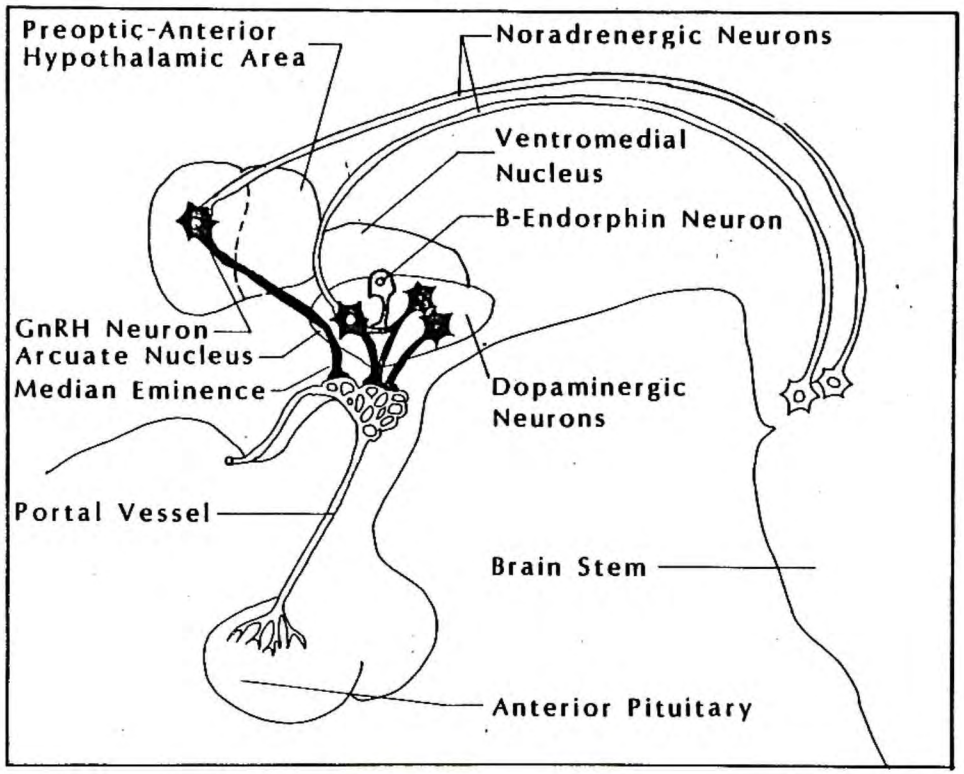
Therefore, pituitary B-endorphin is thought to act as a hormone while that in the brain may act as a neurotransmitter.

B-endorphin is a 31 amino acid peptide with methionine-enkephalin at its N-terminal region which gives it potent opioid activity (Li, 1977). It is cleaved in vivo from B-lipotropin which is originally derived from the precursor molecule, proopiomelanocortin (figure 3; O'Donohue, 1982). Proopiomelanocortin is processed to B-endorphin in the pars intermedia and pars distalis of the pituitary. In the CNS, cell bodies of B-endorphin containing neurons are most concentrated in the medio-basal hypothalamus, particularly in the supraoptic, paraventricular, periventricular, ventromedial, and dorsomedial nuclei with fibers running to other brain structures including the medial amygdula and the paraventricular nucleus of the thalamus (Bloom, 1983).

A number of physiological roles have been suggested for B-endorphin. B-endorphin is thought to be an endogenous analgesic substance and to be more potent than morphine (Alder, 1980) and other endogenous opiates (Loh, 1976). It can effect temperature regulation (Holaday, 1978) and may play a role in the immune response (McCarn, 1982). Along with other endogenous opiates, B-endorphin is thought to suppress appetite, decrease gastrointestinal motility, produce respiratory depression, and effect blood pressure (Faden, 1984).

Figure 4 A schematic drawing of the hypothalamus and pituitary illustrating the close proximity of B-endorphin, dopamine, and GnRH neurons.

NEUROANATOMIC RELATIONSHIPS OF B-ENDORPHIN AND GNRH NEURONS



2. Role of B-endorphin in the Control of the Menstrual Cycle

It has long been known that addiction to opiate drugs such as heroin or methadone is associated with the development of amenorrhea or other menstrual disturbances (Santen, 1973). Recently investigators have unveiled several lines of evidence implicating B-endorphin as having a role in the control of the menstrual cycle. Research concerning these lines of evidence will be discussed in the following review.

a. Anatomical Evidence that Endogenous Opiates are Involved in Gonadotropin Secretion

As previously mentioned, the highest concentrations of cell bodies containing B-endorphin are found in the arcuate nucleus of the medial basal hypothalamus. Also in this area are a high concentration GnRH containing neurons and dopamine, a neurotransmitter thought to modulate gonadotropin release (Ferin, 1984) (figure 4). It has been hypothesized that there is a GnRH pulse generator within the medial basal hypothalamus which receives and integrates neural and endocrine information and translates it into a signal of specific amplitude and frequency. This results in the pulsatile release of GnRH into the hypophyseal portal system and ultimately in the intermittent stimulation of LH and FSH from the pituitary.

Drouva (1981) presented evidence that opiates act through specific opiate receptors on GnRH neurons and

modulate the release of GnRH by an inhibition of the voltage-dependent calcium channels. He studied the rat medial basal hypothalamus in vitro by superfusing the tissue with combinations of a depolarizing dose of potassium, B-endorphin, and naloxone, a specific opiate receptor antagonist. He found that potassium depolarization evoked the release of GnRH and that the addition of B-endorphin blocked this release. Naloxone completely reversed the B-endorphin induced inhibition of GnRH. Neither B-endorphin nor naloxone, in the absence of potassium depolarization, had any effect on GnRH release. Further anatomical evidence for a physiological relationship between the endogenous opiated and GnRH are the findings that the rat axons originating in the arcuate nucleus contain both GnRH and B-endorphin and have been found to terminate in the median eminance near the long portal vessels (Wardlaw, 1980). These authors also documented that there are high concentrations of both substances in the hypophyseal portal blood.

It has also been suggested that B-endorphin acts on the amygdala to inhibit LH release. Parvizi (1980) gave microinjections of B-endorphin into either the basolateral hypothalamus or amygdala of ovariectomized miniature pigs. B-endorphin injected into the amygdala significantly decreased plasma LH levels while injections into the hypothalamus had no effect. The authors conclude that GnRH secretion is altered at the amygdala causing inhibition of pituitary LH secretion.

b. Evidence for Steroid Modulation of the Concentration of Endorphin in the Brain

There are data which demonstrate that both epinephrine and central B-endorphin levels change throughout the menstrual cycle in primates. Plasma B-endorphin levels were measured in five normally menstruating women during the periovulatory period (Laatikainen, 1984). One and two days before ovulation, B-endorphin levels were 4.6 and 4.4 p mol/l respectively. On the day of ovulation B-endorphin concentration rose to 5.2 pmol/l and continued to rise to 8.Ø pmol/l on the day following ovulation. The rises in B-endorphin corresponded to increases in serum estradiol and progesterone.

Ferin (1984) was unable to find a parallel rise in serum B-endorphin and ovarian steroids in monkeys. However, he found marked differences in hypophyseal portal vessel concentration of B-endorphin in monkeys throughout the menstrual cycle (Ferin, 1984B). In intact cycling monkeys B-endorphin levels were undetectable during menstruation, rose during the mid to late follicular phase, then approximately doubled during the luteal phase. When monkeys were ovariectomized, hypophyseal-portal vein levels of B-endorphin were undetectable at any phase of the cycle. Administration of estradiol partially restored B-endorphin levels and a combination of estradiol plus progesterone augmented this steroid effect. Therefore, central B-endorphin concentrations seem to be linked in some manner to circulating steroid levels and steroid feedback at the

hypothalamus.

Injection of naloxone during various phases of the menstrual cycle provides indirect evidence for the role of endogenous opiates in the control of menstrual cyclicity. Intravenous injections of naloxone were administered to normal cycling women during the early and late follicular and the mid luteal phases of their menstrual cycles (Quigley, 1980b). During the early follicular phase, naloxone caused no discernable increase in LH levels. Injections during the late follicular and luteal phases caused marked but distinctly different increases in serum LH levels. In the late follicular phase, naloxone caused a slow steady rise in LH levels. However, during the mid luteal phase, LH values increased rapidly in a pulsatile fashion resembling follicular phase LH patterns. The authors suggest that during the luteal phase, high estradiol and progesterone concentrations feedback to the hypothalamus increasing the LH sensitivity to naloxone as compared to the follicular phase.

c. Evidence that Opiates Decrease Gonadotropin Release

Administration of morphine or B-endorphin suppress LH pulsatility, the preovulatory LH and FSH surges, and ovulation in the rat model. Kinoshita (1980) found that intraventricular administration of 1 microgram B-endorphin in rats suppressed LH pulsatility which was restored by simultaneous intravenous naloxone administration. Likewise,

intravenous morphine injections decreased the magnitude of the LH surge in a dose-dependent fashion and partially prevented the FSH surge (Pang, 1977). Ovulation was completely blocked by morphine but was not affected when rats were pretreated with naloxone. Barraclough (1955) found that chronic treatment with morphine sulfate could delay estrus behavior in rats for up to 25 days.

A recent study by Gabriel (1986) suggests that the endogenous opioid peptides may play a role in determining the magnitude and duration of the steroid induced preovulatory LH surge. He investigated the effect of naloxone on the steroid-induced hypersecretion of LH in ovariectomized rats at various times during the estrous cycle. He found that naloxone was effective in inducing LH hypersecretion prior to and after but not during the LH surge. Therefore, he concluded that there is a decline either in opioid neural activity or in the responsiveness of post synaptic receptors during the preovulatory LH surge in the rat model. In agreement with Gabriel (1986), Allen (1986) found that prolonged opiate inhibition with naloxone prematurely evokes the LH surge in intact rats. He also believes that under normal circumstances endogenous opiate inhibition is removed just prior to the LH surge and that sustained naloxone caused the premature onset of this neuronal event.

Although opiates can not be administered to healthy volunteers for research purposes, there have been numerous reports of menstrual abnormalities among heroin addicts (Wallach, 1964; Stoffer, 1968). In one such study 76 heroin

addicts attending a methadone clinic were investigated and of these only 30% had normal menses. A detailed study of these addicts revealed that four of seven women had no proovulatory LH surge. In these women, plasma progesterone levels remained at follicular levels throughout the cycle and their patterns of LH pulsatility were inappropriate for that particular time in the cycle. These results suggest that heroin was acting centrally to inhibit GnRH release. The resultant LH and FSH levels may have been insufficient for normal follicular develipment resulting in anovulatory cycles.

d. Evidence that Naloxone Restores Gonadotropin Secretion

Both naloxone and naltrexone, administered subcutaneously, or intraperitoneally cause an increase in serum LH in female rats (Cicero, 1979; Bruni, 1977). Evidence that these opiate antagonists were acting at the level of the hypothalamus was provided by the fact that the drugs had no effect on the exogenous GnRH stimulated LH release. The endogenous opioids seem to have a differential effect on LH secretion throughout different phases of the estrous cycle. When naloxone was injected into rats during the 4-day estrous cycle, LH levels were stimulated variably at all times except from 1800 to 2000 hours on the day of proestrous, corresponding to the time of the LH surge (Piva, 1985). The stimulatory effect of naloxone was maximal just prior to the LH surge. Hulse (1983) found that electric

shock stress stimulated-endorphin release eliminated estrous lordosis behavior in rats. As previously mentioned, Gabriel (1986) found a decline in opioid inihibition of GnRH during the LH surge. In contrast, Leadman (1985) observed that intraventricular administration of B-endorphin blocked the preovulatory LH surge and ovulation (Leadman, 1985). In both cases normal estrous behavior and hormone levels were retained with naloxone pretreatment.

In studies of combined groups of men and women, naloxone has been found to enhance (Lightman, 1981; Morley 198Ø) or not change (Jacobs, 1979) serum LH levels, and to increase (Morley, 198Ø) or not change (Lightman, 1981) serum FSH. Naloxone studies in humans provide additional evidence that the level of endogenous opioid inhibition of gonadotropins changes throughout the menstrual cycle. Naloxone is reported to stimulate LH levels during both the late follicular phase and the luteal phase (Blankenstein, 1981; Snowden, 1984). However, no naloxone-stimulated FSH rise has been found during these phases. In most studies, naloxone has failed to change LH levels during the early follicular phase of the menstrual cycle (Quigley, 198Øa, Blankenstein, 1981; Snowden, 1984). However, Moult (1981) found a naloxone induced rise in mean LH level in three females studied during their early follicular phases. In addition, he found a significant increase in LH pulse frequency and a rise in FSH concentrations after naloxone treatment.

Several studies have investigated the effects of endogenous opioids on resting gonadotropin pulsatility. Men,

after receiving naltrexone, had significant increases in both mean LH concentrations and in LH pulse amplitude and frequency (Ellingboe, 1982). These data are in agreement with those of Veldhuis (1984a) who found that an increase in serum dihydrotestosterene accompanied naltrexone-induced augmentation of LH levels in males. One study on eumenorrheic women in their luteal phases found that a six hour naloxone infusion significantly increased both LH pulse amplitude and frequency compared to a control saline infusion trial (Ropert, 1981). Although no FSH pulses could be discerned, there was a significant rise in the mean FSH concentration after naloxone. A second study on estrogen-treated gonadectomized persons with testicular feminization showed that naloxone treatment significantly increased LH pulse frequency measured over an eight hour period (Veldhuis, 1985b).

Taken together, these studies present strong evidence that the endogenous opiates play a role in the control of gonadotropin release in both lower animals and primates, and also in reproductive cyclicity. In addition, it seems clear that the degree of opiate inhibition varies with the changing steroid milieu during different phases of the menstrual and/or estrous cycles.

D. Effect of Exercise on Gonadotropin Pulsatility and GnRH-Stimulated Gonadotropin Release

1. General Overview

In comparison with eumenorrhiec sedentary controls women with exercise-associated amenorrhea have been found to have reduced (Baker, 1981; Loucks, 1984; McArthur, 1980) or normal (Rokainen, 1985a; Chang, 1986; Reame, 1984) basal gonadotropin levels. In addition, earlier studies found that amenorrhea of other etiologies such as weight loss or anorexia nervosa are associated with gonadotropin deficiencies (Vigersky, 1977). These data lead investigators to question whether exercise in some way disrupts normal gonadotropin release.

Pituitary responsiveness to exogenous GnRH has been investigated in eumenorrheic runners, joggers and sedentary controls as well as in males and amenorrheic runners. Two studies administered GnRH to eumenorrheic runners in either the mid-follicular (Boyden, 1984) or late luteal (Ronkainen, 1985b) phase of their menstrual cycles. Boyden (1984) found that basal LH and FSH concentrations were normal in runners at baseline and as well as when they increased their running distances by 30 and then 50 miles per week over a 15 month period. However, GnRH stimulated LH and FSH levels decreased significantly during the peak mileage period, about 75 miles of running per week, compared to a baseline of 15 miles per week. When Rokainen (1985b) studied endurance runners he also found that they had a decreased LH and FSH response to GnRH as compared to joggers or sedentary controls. As in the Boyden (1984) study, baseline concentrations of LH and FSH were normal but runners in this study were found to have

significantly lower serum estradiol and progesterone concentrations compared to their controls.

Amenorrheics have been found to have exaggerated (Rogol, 1984b; Veldhuis, 1985a; McArthur, 1984, Yu-Yahiro, 1986) or normal (Wakat, 1982) LH and FSH responses to GnRH. Rogol (1984b) gave graded doses of GnRH to amenorrheic runners and sedentary controls and found significantly higher LH responses in amenorrheics. Similarly, Veldhuis (1985a) found that amenorrheic runners had higher serum LH after submaximal doses of GnRH as c Amenorrheics have been found to have exaggerated (Rogol, 1984b; Veldhuis, 1985a; McArthur, 1984, Yu-Yahiro, 1986) or normal (Wakat, 1982) LH and FSH responses to GnRH. Rogol (1984b) gave graded doses of GnRH to amenorrheic runners and sedentary controls and found significantly higher LH responses in amenorrheics. Similarly, Veldhuis (1985a) found that amenorrheic runners had higher serum LH after submaximal doses of GnRH as compared to non-runners and that the amenorrheics had normal elevations of serum estradiol in response to the graded pulses of GnRH.

In another study, age and training matched eumenorrheic and amenorrheic long distance runners were compared (Yu-Yahiro, 1986). Both the peak GnRH stimulated FSH and the GnRH stimulated LH area under the curve were significantly greater in amenorrheic than eumenorrheic runners. Basal serum LH and FSH were similar in all runners while serum estradiol was lower in the amenorrheic group. In contrast,

Wakat (1982) studied six oligo-amenorrheic runners and one normally menstruating runner and found that they all had normal responses to GnRH.

Recent studies have demonstrated that amenorrheic runners have abnormal LH pulse frequency (Rogol, 1984b; Veldhuis, 1985a) and pulse amplitude (Fisher,1986). Rogol (1984b) found that six of ten amenorrheic runners had decreased LH pulse frequency (five or fewer pulses in 24 hours) whereas the other four had eleven or more pulses of LH in 24 hour period. Likewise, Veldhuis (1985a) found that six of nine amenorrheic runners had a decreased LH pulse frequency. However, neither the mean LH pulse frequency nor pulse amplitude were different between the amenorrheic group as a whole and a group of sedentary eumenorrheic controls. In each study amenorrheic runners had normal early follicular concentrations of LH, FSH, estradiol, progesterone, and testosterone. Both authors calculated pulsatility by the methods of Santen and Bardin (1973).

Fisher (1986) found no difference in either LH or FSH pulse frequency in amenorrheic runners compared to eumenorrheic runners. However, amenorrheic runners had lower LH pulse amplitudes and lower estradiol, estrone, LH and FSH levels compared to eumenorrheic runners during the early follicular phase of their cycles.

Two studies have investigated gonadotropin pulsatility in eumenorrheic women at rest (Cumming, 1985a) or after an acute bout of exercise (Cumming, 1985b). Blood samples were drawn for pulsatility in eumenorrheic runners and non-

runners over a six hour period during the early follicular phase of their cycles (Cumming, 1985a). Eumenorrheic runners had significantly diminished LH pulse frequency, amplitude, and area under the LH curve compared to controls. When runners performed a 60 minute treadmill run followed by a second six hour pulsatility study, LH pulse frequency was significantly reduced as compared to the testing study. Neither LH pulse amplitude nor LH area under the curve changed as a result of exercise. However, serum LH was significantly higher post-exercise as compared to the pre-exercise values. Cumming determined pulses according to the methods of Reame (1984).

Finally, two studies have investigated gonadotropin pulsatility in male long distance runners. Rogol (1984a) found no differences in either LH or FSH pulsatile release between runners and controls. However, when MacConnie (1986) tested a more highly trained group of runners she found that these men had significantly lower LH pulse frequency and amplitude when compared to age-matched controls. In addition, runners had significantly lower LH responses to graded injections of exogenous GnRH. The authors suggest that elite male runners have hypogonadotropic hypogonadism, similar to what is seen in females with athletic amenorrhea.

In summery, these studies suggest that in female athletes, long distance running can affect normal gonadotropin release. Basal gonadotropin levels and GnRH stimulated gonadotropin release are different in amenorrheic

as compared to eumenorrheic athletes. Possibly, gonadotropin patterns in normally menstruating runners represent a transition to those patterns seen in amenorrheics. Alternatively, it is feasible that in certain susceptible women, an exercise-related disruption in menstruation causes amenorrhea which results in altered gonadotropin release. It also appears that patterns of gonadotropin release change in eumenorrheic runners as they increase their level of training.

E. B-ENDORPHIN AND EXERCISE

1. Introduction

A discussion of B-endorphin levels and exercise must be prefaced by a few notes of caution. First, the measurement of B-endorphin is as yet sub-optimal. Reported recoveries of B-endorphin average about 75%. In addition the antibodies generally used in the B-endorphin radioimmunoassays exhibit at least a 50% cross-reactivity with B-lipotropin. Secondly, few studies have quantitated exercise intensity in relation to B-endorphin secretion. Studies involve both trained and untrained women and usually classify exercise as light, moderate, or heavy without actual quantitation of workload.

A third item to consider is the interpretation of plasma B-endorphin concentrations. Since there are both central and peripheral pools of B-endorphin, it is uncertain whether measurements of venous B-endorphin reflect or influence what

is happening in the brain. This question is of concern since it would seem essential that central B-endorphin activity rises during exercise if endogenous opioids can be implicated in phenomena such as the runner's high and antinociception often reported during exercise, or in changes in GnRH release.

Plasma and hypothalamic B-endorphin levels were measured in rats after foot-shock induced stress (Rossier, 1977). While there was a threefold increase in plasma B-endorphin, no significant difference in hypothalamic B-endorphin was found. However, the author cautions against extrapolating this data to primates since the pars intermedia, the primary source of B-endorphin in rodents, does not exist in primates. Nakao (1980) measured plasma and cerebrospinal fluid B-endorphin in four non-endocrine patients and found no correlation between the two levels. He demonstrated that adrenocorticotropic hormone does not cross the blood brain barrier and suggested that B-endorphin, only slightly smaller in size, may be impermeant as well.

However, it has been suggested that during intense exercise the blood brain barrier may shift its permeability allowing some centrally produced substances into the peripheral circulation (Shangold, 1984). It was demonstrated in the rat model that a nine minute swim in cold water stimulated the release of pituitary B-endorphin and increased the central tissue concentration of endorphin in areas including the amygdala and hypothalamus (Barta,1981). These are sites containing high concentrations of opiate receptors.

Also, Pert (1979) found an increased opiate-receptor occupancy in rats after a short duration run.

Taking these limitations into account, the following is a discussion of the acute and chronic responses of B-endorphin to exercise. Since the exercise-induced responses of B-endorphin to exercise may be influenced by the steroid environment, (Gambert, 1981), this discussion will concentrate on studies performed on women when available.

2. B-endorphin responses to exercise in trained and untrained subjects

Following moderate to high intensity exercise, most studies show a two to threefold increase in B-endorphin in women (McArthur, 1985) and as much as a fivefold increase in men (Berk, 1981). Colt (1981) studied a group of trained men and women who performed two runs, one at a normal training pace and one at near maximal effort. Although B-endorphin rose following both runs, the rise was greater following the strenuous run. Not all runners exhibited an increase in B-endorphin with exercise, however, a greater percentage showed an increase after the strenuous run. Interestingly, the author demonstrated a negative correlation between the percent change in B-endorphin over baseline following exercise and the number of years of training which ranged from four months to sixteen years. Farrell (1982) was unable to show an intensity-dependent increase in B-endorphin in male and female runners who performed treadmill runs for

thirty minutes at 60% and 80% of maximum oxygen consumption. In fact, the post-exercise B-endorphin rise was only significant after the 60% run. However, as in the Colt (1981) study, there was a large intra-individual variation in the B-endorphin response.

The B-endorphin rise with exercise may be augmented following training. Colt (1981) showed that B-endorphin rose 57% following one hour of cycling at 85% of maximum heart rate in sedentary women. Subjects were exercised in a similar manner following one month and two months of very strenuous endurance training and as a result they displayed B-endorphin rises of 79% and 145% respectively. In a study of similar design, B-endorphin was measured following a one hour run consisting of three successive 20 minute runs at 60%, 70%, and 80% of maximum oxygen consumption, performed before training and one and two months following a strenuous exercise program (Howlett, 1984). The authors found a significant rise in B-endordphin post-exercise but no difference in the magnitude of the rise among tests. Both studies were performed in the early follicular phase of the menstrual cycle. Bortz (1981) investigated men and women competing in the Western States 100 mile race. Analysis of B-endorphin was done on samples taken after 60 miles and at the finish of the race. Although B-endorphin rose significantly as a result of the run, the rise was no greater with greater distance run. In fact, B-endorphin levels were lower after 100 miles than after 60.

However, Viswanathan (1985) did find a relationship

between the ambient temperature during exercise and the exercise-induced B-endorphin release. He studied a group of men and eumenorrheic and amenorrheic women who cycled at progressively increasing workloads for 40 minutes to one hour at both 22 degrees Celcius and 5 degrees Celcius. Exercise at 22 degrees Celcius caused a significant rise in B-endorphin in all subjects. At 5 degrees Celcius B-endorphin levels rose in response to exercise in men and eumenorrheic women but the rise in eumenorrheic women was significantly lower compared to that with exercise at 22 degrees Celcius. The exercise-induced B-endorphin rise was completely suppressed in amenorrheic athletes. The authors suggest a sexual dimorphism in the exercise and temperature induced release of B-endorphin and that this response may be altered in amenorrheics.

Gambert (1981) compared the B-endorphin response of untrained men and women to a 20 minute bout of submaximal exercise. While B-endorphin rose significantly in both groups, the rise was much greater in men than women. These data are in agreement with those of Mueller (1980) who showed a larger B-endorphin rise in male rats than female rats after immobilization stress.

In addition, B-endorphin is reported to increase after a power lifting session in weight trained individuals (Elliot, 1984), but not to increase in trained kayakers and untrained controls following isometrics (Melchondia, 1984). Although the isometrics resulted in a 34% decline in strength and in

Table 2 Comparison of recent studies on the B-endorphin response to exercise. The B-endorphin rise may be dependent on type of exercise, sex, and level of training.

TABLE 2

COMPARISON OF RECENT STUDIES ON THE BETA-ENDORPHIN RESPONSE TO EXERCISE

Reference	Subjects	Type of Exercise	% Change Max-Baseline / Baseline
Berk, 1981	6 athletes 6 controls	T-mill max Bruce Protocol	M:208% F:219% M:180% F:171%
Bortz, 1981	51 trained men and women	Western States 100 mile race	@60 mi 185% @100 mi 139%
Carr, 1981	7 originally sedentary women trained for 2 mo.	1 hour stationary cycling @55% max. HR	0 mo.=57% 1 mo.=79% 2 mo.=145%
Colt, 1981	26 trained runners 20 males, 6 females	2 training runs easy and hard	easy 49% hard 241%
Gambert, 1981	5 men, 4 women untrained	20 min T-mill run @ 80% max HR	M: 366% F: 200%
Farrell, 1982	trained runners, 5 men + 1 woman	30min.runs at 60% + 80%	60%max=N.S. 80%max=N.S.
Elliot, 1984	5 males untrained	max T-mill test max lifts on universal gym	T-mill-95% weights-150%
Howlett, 1984	15 women originally sedentary trained for 2mo	T-mill run 1 hr 20 min ea @ 60% 70%+80%VO2 max	0 mo.=700% 1 mo.=400% 2 mo.=480%
Janal, 1984	12 trained male runners	6.3 mile run 85% VO2 max	66%
Melchionda, 1984	6 trained kayakers, 14 untrained men and women	isometric knee extension	N.S.

severe reported discomfort, B-endorphin levels actually decreased. B-endorphin levels do not appear to correlate with perceived exertion or length of training session.

Janal (1984) also did not find a correlation between perceived exertion and pain and increases in B-endorphin following a 6.3 mile run at 85% of maximum aerobic capacity in trained male runners. Table 2 summarizes the results of the studies cited above.

Finally, the effect of training female Wistor rats on a treadmill daily for eight weeks was assessed and compared to a sedentary control group (Blake, 1984). After the eight weeks half of the trained and half of the control rats were exercised to exhaustion and decapitated. The other half was not exercised before decapitation. In both groups the fatiguing run resulted in a decrease in serum LH and an increase in B-endorphin in the nucleus accumbens. In addition, the amygdala had increased leu-enkephalin levels in trained rats and these levels were higher in non-fatigued than fatigued rats. The authors conclude that in this animal model there are changes in brain endorphins in response to acute, fatiguing exercise.

Therefore, the rise in serum B-endorphin following exercise seems to be greater in males than females. The greater the intensity of exercise, the greater the increase in B-endorphin. There does not seem to be a positive correlation between the post-exercise rise in serum B-endorphin and the years of training or the duration of the training session.

F. EFFECTS OF ACUTE AND CHRONIC EXERCISE ON HORMONES THAT MAY INFLUENCE NORMAL REPRODUCTIVE FUNCTION

Aerobic exercise induces a variety of acute and chronic physiological adaptations. A review of the effects of aerobic exercise on oxygen consumption, percentage of body fat, and caloric expenditure may aid in an understanding of the humoral responses to exercise. At submaximal workloads, oxygen consumption increases linearly with an increased intensity of exercise. When sufficient oxygen is available one molecule of glucose, via aerobic metabolism, is processed through a series of biochemical reactions in glycolysis, the Kreb's cycle, and the electron transport system, to produce 38 molecules of ATP. The energy for muscle fiber contraction during exercise is supplied by the ATP. If a sufficient supply of oxygen is not available to meet the muscle's metabolic demands, glycolysis will proceed anaerobically resulting in the conversion of pyruvate to lactic acid and the production of only 2 ATP per glucose molecule. A large accumulation of lactic acid can cause metabolic acidosis and cessation of activity. Therefore, at heavy workloads, the higher one's oxygen consumption, the more efficient will be one's use of available energy stores, and the greater will be the amount of ATP available for muscular work.

Maximum oxygen consumption (VO2 max) is an index of a person's physical fitness level and is known to increase with regular aerobic activities such as swimming, cycling, and endurance running. The maximum oxygen consumption in a sedentary female is 25 to 35 milliliters of oxygen per killogram of body weight per minute (ml/kg/min). Top female endurance athletes have a maximal oxygen uptake that is about twice that of sedentary persons (Astrand, 1977), while olympic athletes have reported maximal oxygen consumptions as high as 75 ml/kg/min.

Approximately half of this increase in VO2 max with training is attributed to an increase in cardiac output due primarily to a greater stroke volume, the result of cardiac hypertrophy. Accounting for the other fifty percent of the increase is a greater oxygen extraction by muscle cells which lowers the oxygen tension in the blood and increases the arterial-venous oxygen difference.

The biochemical adaptations which allow more oxygen to be extracted from the blood are numerous. There is an increase in skeletal muscle myoglobin which speeds the rate of oxygen transport through the fluid layer and may make the diffusion rate of oxygen through the cytoplasm to the mitochondria more rapid (Gollnick, 1969). Secondly, there is an increase in the size and number of the mitochondria as well as an increase in some mitochondrial enzymes of the Kreb's cycle and electron transport system (Holloszy, 1973). Endurance athletes have a higher percentage of the oxidative, slow twitch fibers than do power athletes or sedentary

subjects (Rusko, 1978). Some believe that there is a conversion of glycolytic fast twitch fibers to slow twitch fibers with endurance training (Morgan, 1971).

One liter of oxygen consumed is approximately equal to five kilocalories of energy expended. Physical activities of varying intensities can be classified in metabolic units, or METS. A MET is defined as a multiple of the resting metabolic rate of a sedentary individual. The resting oxygen consumption for a woman is about 200 milliliters per minute and for a man approximately 250 milliliters per minute (McArdle, 1981). For a task of a given intensity, the greater one's body weight the greater will be the amount of energy expended. Therefore, a MET can also be expressed in terms of oxygen consumption per kilogram of body weight, 1 MET equal to 3.6 ml oxygen/kg/min.

As an example, for an average 55 kilogram woman, a light activity such as standing at the ironing board ironing would require between 1.2 and 2.7 METS. A moderate activity like leisure cycling would require 4.4 to 5.9 METS. Running on a horizontal surface such as the treadmill at a pace of 8 minutes per mile, the average pace of the women in this study, would require greater than 10 METS or an expenditure of approximately 12 kilocalories per minute.

Under basal metabolic conditions the respiratory quotient (R.Q.), the ratio of carbon dioxide produced over oxygen consumed, is an index of the type of nutrient being catabolized. For example, the R.Q. for carbohydrate is

1.ØØØ, for fat is Ø.696 and for protein is Ø.818. During submaximal exercise an increase in oxygen consumption is accompanied by a proportional rise in carbon dioxide elimination. The point at which oxygen consumption has reached its maximal capacity and plateaus but carbon dioxide continues to rise is called the anaerobic threshold. At this point, hyperventilation occurs and the amount of carbon dioxide produced is disproportionate to the metabolic demands of exercise. This ratio during exercise is referred to as the respiratory exchange ratio (McArdle, 1981). When this ratio of carbon dioxide consumed increases above 1.ØØØ it indicates anaerobic metabolism.

1. Reproductive Hormone Responses to Acute Exercise

A variety of hormonal changes have been reported to occur in response to acute exercise in both runners and sedentary controls. Estradiol and progesterone increase in response to exercise and the increase in these steroids is greater with a greater exercise intensity (Bonen, 1984). The exercise-induced rise in progesterone occurs only in the luteal phase, not the follicular phase (Jurkowski, 1978; Bonen, 1983) and this rise is significantly less in trained women as compared to controls (Bonen, 1983).

Jurkowski (1978) studied nine untrained females during both the follicular and luteal phases who exercised at low, moderate, and exhaustive exercise loads. He found that estradiol increased significantly at all workloads during the

luteal phase but only after exhaustive exercise in the follicular phase. Another study showed that follicular phase serum estradiol increased 107% in women after running a marathon (Pepper, 1983). Spitler (1983) showed a significant increase in total estradiol after short term intense exercise (85-90% VO2 max) but no significant rise following 90 minutes of moderate exercise (50% VO2 max) in trained women during their early follicular phase. Loucks (1984) found no increase in either estrone or estradiol following a 40 minute run at 80% of maximum oxygen consumption.

Keizer (1980) studied the metabolic clearance rate of estradiol in six untrained women who biked for ten minutes at 75% of maximum oxygen consumption. He reported a 36% decease in the metabolic clearance rate of estradiol. Bonen (1984) also showed a decrease in the metabolic clearance rate of estradiol that was intensity dependent. She points out that plasma steroid levels represent the sum of glandular secretion, peripheral conversion and metabolic clearance. Decreased metabolic clearance of the steroids can account for some but not all of the exercise-induced rise since the increases are also dependent on the phase of the menstrual cycle. In addition, a post-exercise increase in estrone was found in both runners and non-runners (Cummings, 1983).

Testosterone levels increase almost twofold in trained runners post-exercise (Shangold, 1981). The exercise induced rise is much greater in the follicular (51%) compared to the luteal phase (21%). Androgens (70% dihydrotestosterone + 100% testosterone) also increased significantly in untrained

women following a 3Ø minute treadmill walk (Wallace, 1983). In addition, a rise in both total testosterone and non-sex hormone binding globulin bound testosterone was found in women after anaerobic resistance exercise (Wall, 1983). However, one study found no change in serum testosterone in trained runners after a hard 4Ø minute run (Loucks, 1984).

In two studies, when corrected for exercise stimulated changes in hematocrit, no difference in LH was observed following exercise in either the luteal or follicular phases (Bonen, 1983; Jurkowski, 1978). One of these studies found a significant exercise-induced rise in FSH only in the follicular phase (Jurkowski, 1978). Cumming (1983) reports a significant increase in FSH in both trained and untrained women following 6Ø minutes of exercise. He also found a pre-exercise anticipatory rise in LH which persisted through the first 15 to 2Ø minutes of exercise in both groups of women. In addition, Hale (1983) found a 36% increase in LH levels in women after running a marathon, but no change in FSH levels.

2. Reproductive Hormone Responses to Chronic Exercise

Daily bouts of endurance exercise over an extended period of time cause a number of subtle hormonal changes and alterations in the menstrual cycle patterns of eumenorrheic athletes. Studies investigating these changes have been of both cross-sectional and longitudinal design and have studied subjects including swimmers, ballet dancers, and long and middle distance runners. Due to differences in training

regimens and in some cases to poor experimental design, the results of these studies are for the most part conflicting, offering no general consensus on the long term effects of training on hormonal status.

Resting levels of luteinizing hormone have been reported to increase (Bonen, 1984) decrease (Russell, 1984b; Dale, 1979a & b), or not change (Shangold, 1979) with endurance training. Bonen studied four teenaged competitive swimmers and found the swimmers to have significantly elevated LH levels and depressed FSH levels compared to four age-matched controls. In contrast, another group of teenaged swimmers and a group of marathoners had lower LH levels than the controls (Russell, 1984b). Mean values for FSH were similar for all three groups. Furthermore, in a case study no differences were seen in luteal phase LH or FSH levels as a result of 18 months of marathon training (Shangold, 1979).

Female long distance runners are reported to have both increased (Dale, 1979a) and decreased serum testosterone levels (Ronkainen, 1985a). Dale found that long distance runners had significantly higher testosterone values than controls (Ø.41 vs. Ø.33 ng/ml) and that joggers had intermediate values (Ø.38 ng/ml). He pointed out that all testosterone values were within normal physiololgical limits and suggested that higher androgen levels may be advantageous to runners due to their anabolic effects. He hypothesized that higher androgen levels can be explained by stress induced adrenal cortex production or possibly may be due to

decreased aromatization of androgens to estrogens in these lean individuals. Ronkainen (1985a) compared runners during their hard training and light training seasons and found significantly lower serum testosterone values during ovulation and in the luteal phase of the hard training season.

Serum estradiol levels tend to be lower in trained runners and swimmers compared to controls throughout the menstrual cycle (Dale, 1979a) during the follicular phase (Bonen, 1984) and during the preovulatory surge. In addition, there are reports of both increased (Rebar, 1981) and decreased (Schwartz, 1981) estrone to estradiol ratios in endurance runners.

A number of studies however, agree that luteal phase progesterone production is low in women participating in endurance training. In light of these findings recent reports of shortened luteal phase lengths in athletes are of special interest. One study found that, of the subjects studied, only 50% of the runners compared to 67% of the joggers and 83% of the controls had luteal phase progesterone levels greater than 2 ng/ml (Dale, 1979a). When the runners with low luteal phase progesterones were investigated for anovulation, none were found to have preovulatory LH or FSH peaks. A group of swimmers had low luteal phase progesterone values despite the presence of an LH surge and this suggested anovulation and/or nonluteinization of the follicle (Bonen, 1981). These swimmers also had shortened luteal phase lengths of five days or less. Shangold (1979) found that mid

luteal progesterones were lower in training than non-training cycles and that the luteal phase length was inversly proportional to the total distance run during the follicular phase. Luteal phase length was 13 to 14 days long in cycles in which the subject ran five miles/week or less and were nine days or fewer in cycles with greater than 35 miles/week of running. There was no difference in total cycle length.

Prior (1982) studied 48 cycles by basal body temperature in 24 women training for a marathon. She found that 32 of the cycles were biphasic and 16 were monophasic indicating anovulation. Of the biphasic cycles, 16 had a luteal phase length of 11.1±1.2 days, within normal limits, and the remaining 16 had shortened luteal phase lengths averaging 6.4±Ø.9 days. The average training distance was significantly shorter in the normal biphasic group (7.9±2.4 mile/week) compared to the shortened luteal phase (9.6±1.8 mile/week) and monophasic cycle (9.9±1.8 mile/week) groups.

In a prospective, longitudinal study, 3Ø formerly sedentary women participated in an intense running and exercise program for three to five hours per day, over two menstrual cycles (Bullen, 1985). During the training period 14 (46.7%) reported intermenstrual bleeding, 13 (43.4%) had delayed menses, and 18 (6Ø%) had loss of LH surge accompanied by a shortened luteal phase length. All of the women who ceased exercising after the study resumed normal menses.

Casper (1984) used midluteal intravenous isoproterenol infusions to simulate the cardiovascular effects of a hard

training run in six sedentary eumenorrheic women. He found a dramatic decrease in estrogen and progesterone levels, 30% and 50% respectively, but no change in serum LH. He hypothesized that chronic exercise increases the metabolic clearance rate of steroids, which over time, could lead to disruption of the normal positive or negative feedback of steroids on the hypothalamic or pituitary level.

3. The Cortisol Response to Exercise

Exercise is a potent stimulus of cortisol release and this relationship is intensity dependent and correlates with corresponding increases in adrenocorticotropic hormone (ACTH) (Farrell, 1983). Moderate exercise may not be sufficient to change glucocorticoid levels (Tharp, 1975; Shepherd, 1975) but intense exercise causes cortisol to rise as much as fourfold (Semple, 1985). In fact, Few (1974) found that cortisol levels decreased after "light" exercise but increased markedly when exercise load exceeded 65% of VO2 max. He hypothesized that exercise itself stimulates an increase in the rate of cortisol uptake by peripheral tissues. When the workload exceeded a critical level it triggered a massive secretion of cortisol by the adrenal cortex.

Cortisol plays an important role in supplying energy to the working muscles during exercise. It affects carbohydrate production by promoting synthesis of hepatic enzymes involved in gluconeogenesis thereby elevating hepatic glucose production. In addition, cortisol stimulates the release of

muscle alanine which is an important gluconeogenic substrate. It blocks glucose uptake by peripheral tissues, and increases plasma fatty acid concentrations by promoting lipolysis (Appenzeller, 1983). Cortisol also has anti-inflammatory actions and repeated glucocorticoid release with chronic exercise results in adrenal gland enlargement (Terjung, 1979).

Few studies have examined the effect of exercise on cortisol levels in women in relationship to their reproductive endocrine status. One study looked at cortisol levels in amenorrheic runners and in eumenorrheic runners and non-runners in the early follicular phase of their cycles in response to graded maximal bicycle exercise. Cortisol initially declined in non-runners but not in runners. However, cortisol rose significantly at maximal exercise with no difference among the groups in the magnitude of the rise (Cumming, 1983). In contrast, Loucks (1984) found a significant cortisol rise in eumenorrheic runners after 40 minutes of treadmill exercise at 80% VO2 max, but not in fitness matched amenorrheic runners after the same bout of exercise. In this study prolactin and androstenedione also increased post-exercise in eumenorrheics but not amenorrheics. Two other studies have examined the cortisol response to exercise in mixed groups of untrained men and women. Both of these studies found increases in plasma cortisol after maximal bicycling (Maehulum, 1986) and treadmill running (Farrell, 1983) and these elevations

persisted for one hour post-exercise.

a. The Effect of Elevated Cortisol Levels on Normal Reproductive Function

Increased adrenal activity adversely affects the normal release of reproductive hormones in a variety of animal models. In both male and female rats chronic stress, due to either daily immobilization, cold exposure, or exercise, caused reduced circulating levels of LH, testosterone and prolactin. In contrast, bouts of acute stress caused temporary rises in adreocorticotropic hormone, prolactin and luteinizing hormone (Collu, 1979). Also, in male hypophysectomized rats, either dexamethasone or corticosterone administered concomitantly with FSH caused a decrease in testicular LH receptors (Bambino, 1981). Electroejaculation stress induced a rise in cortisol in male cheetahs but no change in LH or testosterone levels (Wildt, 1984). In contrast, electroejaculation stress in bulls caused an increase in cortisol and a temporary decrease in both LH and testosterone secretion. The author suggests a physiological integration of adrenal and testicular endocrine function in this species (Welsh, 1981). However, electroejaculation stress caused no change in serum cortisol, LH, or testosterone in the male clouded leopard (Wildt, 1986). Wildt (1986) explains that the response of the adrenal-pituitary-testicular axis is species specific and that, depending on the species, stress may induce an increase, decrease, or no change in LH or testosterone

levels. Perhaps this is also true for the hypothalamic-pituitary-ovarian axis as well.

In adult males, oral administration of either cortisol or dexamethasone, completely suppressed the nocturnal rise of testosterone. Serum LH and FSH did not decrease during the study period and in fact, rose somewhat at night compared to the gonodotropin levels in control subjects. The authors suggest that the observed decline in testosterone was LH independent and due to a direct effect of cortisol on testosterone biosynthesis (Doerr, 1976).

Both Baker (1982b) and Cumming (1983) have demonstrated a simultaneous secretion of cortisol and androgens in eumenorrheic women in response to exercise stress. Loucks (1984) had similar finding in her eumenorrheic runners but found no exercise-induced increase in either cortisol or androstenedione in amenorrheic runners after exercise. She suggests that stress might alter the normal androgen concentration or androgen estrogen ratio and thereby disrupt normal regulation of the female reproductive system.

Therefore, cortisol appears to acutely alter reproductive steroid levels in humans. If exercise induces daily elevations in cortisol, this may eventually result in altered steroid levels and disruption of steroid functions.

G. EXERCISE ASSOCIATED AMENORRHEA

Athletic amenorrhea has been classified as a type of "chronic anovulation syndrome", which is a kind of secondary amenorrhea. Secondary amenorrhea refers to loss of menstrual cycles for a period of more than six months. In contrast, women with primary amenorrhea have never had a menstrual cycle. Since there is no known physiological mechanism for exercise-associated amenorrhea, it is not certain whether it is caused by a single factor or through the synergy of a number of factors, and whether it is a distinct entity or a variety of conditions which share common symptoms. Part of the confusion about this condition arises from the failure of researchers in this field to reach a consensus on the definition of athletic amenorrhea. In a number of studies, athletes were considered as amenorrheic if they had had no menstrual periods for a time ranging from three months to one year. Additional problems with these studies include failure to control for use of oral contraceptives and to separate athletes in aerobic-type sports from those doing anaerobic activities. Lastly, in many of these studies amenorrheic athletes were not distinguished from those with oligomenorrhea, and women with primary amenorrhea were grouped with secondary amenorrheics for study purposes.

1. Incidence

The incidence of secondary amenorrhea in athletes has been reported to be from 3.5% (Lutter, 1982) to 44% (Calabrese, 1982) compared to the incidence in the general population which ranges from 1.8% to 5% (Petterson, 1973;

Singh, 1981). Menstrual disorders in female athletes have been documented through a variety of self-report type studies. For instance, a study of top Norwegian athletes from 27 different sports revealed a 10.1% incidence of amenorrhea. Lutter (1982) surveyed 350 female long distance runners and found that 3.4% were amenorrheic and 19% had oligomenorrhea. Shangold (1982) also found a high incidence of amenorrhea/oligomenorrhea among 394 participants in the New York City Marathon. However, a more indepth study revealed that of the original 24% with reported menstrual irregularities, 19% had had these irregularities prior to the onset of training. Foreman (in Bloomberg, 1977) distributed questionnaires to 47 runners at the 1971 and 1973 National AAU Women's Cross Country Championships. He found that 27 runners had regular periods while nine had irregular periods and eleven menstruated very irregularly, no more than two times per year or not at all. Also Speroff (1980) reported that 7% of 872 runners answering a newspaper advertisement were amenorrheic and Carlberg (1981a & b) found that 12% of 2523 varsity athletes had oligo/amenorrhea.

Menstrual abnormalities are also common among ballet dancers. When thirty-four dancers from a single school were interviewed, 44% reported amenorrhea and 50% olilgomenorrhea (Calabresse, 1982). Similarly, Frisch (1980) discovered that 25% of 89 dancers studied were amenorrheic, 40% of these reporting primary amenorrhea. In addition another 30% of the dancers were oligomenorrheic. Amenorrhea has also been cited

in swimmers (Frisch, 1981), gymnasts (Baker, 1981), and basketball players (Webb, 1981).

2. Proposed Contributing Factors

a. Body Fat

The percentage of body fat, total body weight, and changes in body weight are all thought to contribute to the functioning of the menstrual cycle. Wilmore (1975) states that, in general, the female endurance athlete is of average height, slightly lower total body weight, lower fat weight, and slightly higher lean body weight as compared to her sedentary counterpart. A 10-15% weight loss or a decrease of about one third of one's total body fat has been shown to result in amenorrhea (Dale, 1979A). While Frisch (1976) states that the relative percent body fat for American girls ranges from 22-24%, Wilmore describes the body composition of the average female distance runner to range from 11-15%. This is interesting in light of the findings of Frisch (1974) that a 17% body fat level is necessary for the onset of menarche while 22% body fat is required for menstrual cycle maintenance.

Comparisons of body composition for amenorrheic and eumenorrheic athletes have been contradictory. Some studies have found amenorrheic athletes to have a significantly lower percent body fat than eumenorrheic athletes (Feicht-Sanborn, 1982; Schwartz, 1981; Speroff, 1980). However, both Warren (1980) and Baker (1981) have reported the onset of amenorrhea

in athletes without a change in body weight or body fat. Still others have found no significant difference in percentage of body fat between eumenorrheic and amenorrheic athletes (Baker, 1981; Calabrese, 1982; Dixon, 1984; Yu-Yahiro, 1983).

The loss of body weight due to exercises may also contribute to the onset of menstrual irregularities. Amenorrheic runners had significantly greater weight losses than normally menstruating runners (Schwartz, 1981; Dale, 1979b; Speroff, 1980). These authors also found that lower body weight for height prior to training was a predictor for the development of menstrual irregularities.

b. Training Intensity

Athletic amenorrhea is most prevalent in women performing endurance-type sports such as long distance running. Amenorrhea may be positively correlated to the number of miles run in training. Foreman (in Bloomberg, 1977) surveyed female intercollegiate athletes and found that only long distance runners and cross country skiers had trouble with their menstrual cycles. Similarly, Erdelyi (1976) found a higher incidence of menstrual irregularities in athletes in endurance sports compared to girls participating in power-type activities.

Two studies have found that the development of menstrual irregularities was related to the number of miles run per week. Feicht (1978) surveyed track and field participants

and found that only 6% of those who ran the least had menstrual problems while 43% of women running 50 miles or more per week developed menstrual abnormalities. Also Dale (1979b) found that the average number of menses per year for his marathon and jogging groups were 7.8 and 8.5 respectively as compared to 11.5 menses per year in the sedentary controls. Neither Speroff (1980) nor Baker (1981) were able to find a relationship between distance run and the lack of normal menses.

c. Menstrual History

Menstrual irregularities seem to be higher in young athletes, especially those that began training prior to menarche, while menstrual irregularities are lowest in older athletes and in those who have been pregnant. Dale, (1979A) found that 50% of his runners who had never been pregnant were oligomenorrheic, while only 20% of those runners who had previously been pregnant had oligomenorrhea. He suggests that pregnancy is an index of hypothalamic maturity which could decrease a runner's susceptibility to the development of menstrual problems.

Baker (1981) found that runners less than 30 years of age had a higher incidence of amenorrhea than those over 30. In this study the mean age of amenorrheics was 24.3 years and that of eumenorrheics was 31.4 years. Likewise Kyle (1986) surveyed participants in the First Women's Olympic Marathon Trials and found that amenorrheic women were significantly younger than eumenorheics (25.6 vs. 30.7 years old). These

data are in concordance with Speroff (1980) who found that women runners under 25 years old were much more likely to develop amenorrhea than older runners.

In addition, women endurance athletes and especially ballet dancers, tend to have a later age of menarche than nonathletes. Frisch (1981) studied two groups of college swimmers and runners, those who began training before menarche and those who started training after menarche. She found that pre-menarche trained runners had a mean menarcheal age of 15.1 years compared to menarcheal ages of post-menarche trained and sedentary controls of 12.8 and 12.7 years respectively. Twenty-two percent of the pre-menarche-trained athletes were amenorrheic while none of those who initiated training after menarche had amenorrhea.

Calabrese (1982) found that the mean age at menarche in a group of ballet dancers was 14.3 years. Similarly, Frisch (1980) found that the mean age at menarche of 67 post-menarchal dancers was 13.7 years. Nine of the dancers she studied (mean age 18.5 years) had not yet attained menarche.

d. Nutritional Status

Changes in nutrient intake as well as the increased caloric expenditure that accompany exercise may contribute changes in to menstrual status in female athletes. Frisch (1981) reported that premenarche trained runners, most of whom were amenorrheic or oligomenorrheic, ingested less protein, fat, and calcium, and less total calories than post-

menarche trained runners. Likewise, Nelson (1985) found that amenorrheic runners had significantly lower intakes of carbohydrates, total calories, and fat as compared to eumenorrheic runners. Calcium intake was sufficient in both groups of runners but 82% of amenorrheics and 35% of eumenorrheics consumed fewer calories than the U.S. recommended daily allowances. Also Schwartz (1981) found that the diets of amenorrheic athletes consisted of a significantly lower percentage of protein compared to eumenorrheic runners. A group of ballet dancers were found to consume an average of 1360 kilocalories per day, only 71.6% of the Recommended Daily Allowance for women of their age and height (Calabrese, 1982). In addition to the caloric deficit, more than half of the dancers were deficient in vitamins D and B12, folic acid and iron. Also there have been reports of increased vegetarianism among amenorrheic athletes (Appenzeller, 1983).

In contrast, some have found no differences in nutritional status between amenorrheic and eumenorrheic athletes. Kyle (1986), investigated exercise-induced changes in blood minerals and associated proteins in amenorrheic and eumenorrheic marathoners. She found no differences between groups either at rest or after running a marathon. Likewise, analysis of serum of fats, proteins, and minerals revealed no differences between amenorrheic and normally menstruating runners (Dale, 1979a; Appenzeller, 1983).

e. Psychological Stress

Psychological or emotional stress is believed to contribute to some types of non-athletic amenorrhea such as anorexia nervosa. Therefore, it has been hypothesized that the stress of competition or increased time committments involved in training may be related to the onset of athletic amenorrhea. Schwartz (1981) tested this theory and found that amenorrheic runners associated more subjective stress with their running than did normally menstruating runners. However, standard psychological tests revealed no differences in stress levels between the groups and all responses were within the normal limits of the tests.

3. Humoral Profile

Both the acute and chronic endocrine responses of cyclic women to exercise have been discussed in a previous section. This review will focus on studies which describe how the basic endocrine profile of the amenorrheic athlete differs from that of the cyclic athlete and sedentary control.

a. Gonadotropin Levels

Basal gonadotropins have been measured in several groups of amenorrheic athletes and dancers with conflicting results. Luteinizing hormone has been found to be lower in amenorrheic athletes compared to eumenorrheic athletes (Baker, 1981; Loucks, 1984) or eumenorrheic controls (Baker, 1981; McArthur, 1980; Laatikainen, 1986). Several studies have

found no differences in basal LH levels between amenorrheic and eumenorrheic runners (Yu-Yahiro, 1986; Horgan, 1983; Carlberg, 1981b) and still others have found basal LH to be elevated in amenorrheic athletes (Schwartz, 1981; Bonen, 1981; Brisson, 1982). Oian (1984) and Loucks (1984) found that amenorrheic athletes had clinically low FSH levels. However, most other studies have found normal FSH levels in cyclic athletes, even in the presence of abnormal LH values (McArthur, 1980; Baker, 1981, Schwartz, 1981).

The LH and FSH responses to a bolus injection of naloxone were no different between amenorrheic and eumenorrheic runners (Dixon, 1984). In response to an acute bout of exercise LH and FSH levels were unchanged in amenorrheic athletes (Carlberg, 1981a; Fears, 1982). However, Cumming (1983) found a post-exercise increase in LH and FSH levels in both amenorrheic and eumenorrheic runners.

b. Steroids

Most studies have found that amenorrheic athletes have below normal (Yu-Yahiro, 1986; Baker, 1981; Dixon, 1984; Horgan, 1983; Oian, 1984; Laitikanen, 1986; Loucks, 1984) or normal early follicular phase levels of estradiol (Schwartz, 1981; Carlberg, 1981a). Progesterone levels are in the normal range for the early follicular phase of the cycle. Elevated estrone to estradiol ratios were found in oligomenorrheic athletes (Carlberg in Appenzeller, 1983) and in both amenorrheic and eumenorrheic runners when compared to sedentary controls (Schwartz, 1981). The authors suggest that peripheral steroid metabolism may be abnormal in

athletes with menstrual disorders.

Androgen excess is associated with menstrual abnormalities under a variety of circumstances, such as polycystic ovarian disease. Initial findings of slightly elevated basal serum testosterone (Dale, 1979B; Oian, 1984; Carlberg, 1981b) and exercise-induced increases in serum testosterone (Kuoppasalm, 1976) raised the possibility that transient or persistent hyperandrogenism might cause amenorrhea. However, subsequent studies have found that in response to exercise, serum testosterone rises in both eumenorrheic and amenorrheic athletes with no differences in the magnitude of the rise (Cumming, 1983; Shangold, 1981; Baker, 1981b). In addition, some studies have found slightly elevated dihydrotesterone (Oian, 1984; Carlberg, 1981b) and dehydroepiandrosterone (Baker, 1981) in amenorrheic athletes.

c. Other Hormones

In clinical states, hyperprolactinemia is a well recognized cause of amenorrhea. However, studies have found basal prolactin levels to be either normal (Fears, 1982; Bonen, 1981; Schwartz, 1981; Cohen, 1982) or low (Baker, 1981; Laatikanen, 1986) as compared to eumenorrheic athletes or controls. A number of studies have reported that female runners show a rise in serum prolactin after acute exercise, but sedentary women (Brisson, 1980; Chang, 1986; Shangold, 1981; Baker, 1981b) and amenorrheic women (Loucks, 1984) do not. However, some of these studies and others (Fears, 1982;

Wakat, 1982) have shown no differences in the exercise-induced prolactin rise between amenorrheic and eumenorrheic athletes.

Most studies have found amenorrheic athletes to have normal thyroid function (Yu-Yahiro, 1986; Oian, 1984; Dale, 1979A) although one study found elevated thyroid stimulating hormone levels in amenorrheic runners (Schwartz, 1981). In addition, amenorrheic athletes are reported to have normal resting (Fears, 1982; Baker, 1981) as well as post-exercise (Yu-Yahiro, 1986) cortisol levels as compared to eumenorrheic athletes.

H. JUSTIFICATION FOR THIS RESEARCH PROJECT

The humoral response to exercise is impacted upon by a multiplicity of physiological, psychological, and environmental stresses. It is not possible in a single study to investigate all of the exerscise-related parameters that may lead to the development of menstrual disorders in female athletes. However, a review of the literature suggests the need for a well controlled study which investigates gonadotropin pulsatility in elite female athletes. Also, recent studies demonstrate that B-endorphin levels increase as a result of exercise and suggest that B-endorphin may play a role in control of GnRH release. This relationship of B-endorphin to gonadotropin pulsatility had never been explored. Furthermore, the prevalence of studies on women with exercise-associated amenorrhea and their failure to conslusively establish a mechanism by which amenorrhea

develops, suggested a need for a novel research approach.

Therefore, this study investigated eumenorrheic runners compared to eumenorrheic sedentary controls to determine whether any degree of abnormality in pulsatile gonadotropin release could be demonstrated and to identify some of the premorbid factors that could signal disruption of normal menstrual cyclicity. In addition, this study sought to determine whether B-endorphin levels at rest and/or after exercise were related to alterations in gonadotropin release.

III. METHODS

A. Subject Selection

Subjects included two groups of women: nine eumenorrheic sedentary controls and nine eumenorrheic highly trained long distance runners. All women were between the ages of 23 and 43 and had had normal menstrual bleeding every 23 to 36 days within the past year. None of these women had ever received medical treatment for infertility.

Subjects were solicited by phone and through notices placed on bulletin boards at the Uniformed Services University and at two local running stores. In addition ads were placed in the NIH Record and the newsletters of two local running clubs. Women who responded to the ads were given an initial phone interview to determine eligibility for the study.

Subjects included in the study were non-smokers and had not taken birth control pills within the last ten months. Excluded from the study were women who considered themselves to be more than ten pounds overweight, or those who had had a weight gain or loss of more than ten pounds within the past three months. Those persons taking medications other than oral contraceptives were screened by a physician during part I of the study and eliminated only if her medication(s) would effect the outcome of the study.

During the phone interview subjects were also questionned about their physical activity habits. Sedentary controls

were not engaging in physical activity requiring an energy expenditure of greater than 3 METS more than one time per week. Runners included women expending more than 9 METS for 7.5 hours per week of which 5.25 METS (equal to 35 miles of running per week) was spent in running.

Once a subject was determined to be eligible for the study, she was sent a packet containing a volunteer agreement, approved by the Human Subject Committee at the Uniformed Services University and explaining in detail what would be required of each subject. Menstrual and physical activity questionnaires designed by the investigator for this study were also included as was the General Services Administration's standard form 93, Report of Medical History. Subjects were instructed to read all forms carefully and to complete them before their laboratory visit.

B. Experiments

1. Part I: Baseline Data Collection

Subjects fasted for twelve hours before reporting to the laboratory between Ø73Ø and Ø93Ø. Upon arriving a physician gave each subject a brief physical examination to screen for medical problems that would preclude performing an exercise test. Among other things this included listening to the heart and lungs and taking resting blood pressure. Both the investigator and the physician reviewed the questionnaires

and medical history and the General Services Administration standard form 88, Report of Medical Examination was completed by the physician for each subject during the patient interview. Height without shoes was measured in centimeters and dry land weight was taken in kilograms while subjects were wearing only light weight running shorts and a shirt. In all but one case subjects were cleared for further participation in the study. One subject was eliminated from the study due to obesity.

a. Resting Blood Sample

A fasting resting blood sample was drawn from the antecubital vein for determination of hematocrit, and plasma concetrations of estradiol(E2), progesterone(P), testosterone(T), luteinizing hormone(LH), follicle stimulating hormone (FSH), cortisol (C), and Beta-endorphin/Beta-lipotropin immunoreactivity (B-end). The sample was placed in tubes containing liquid Ethylenediamine tetraacetic Acid (Ø.5 ml of 1Ø% EDTA), mixed by inversion for 3Ø seconds, and placed in ice. Resting blood samples were drawn between Ø8ØØ and Ø93Ø to control for the diurnal variations of the hormones.

b. Skin Preparation and Electrode Placement

A twelve lead electrocardiogram (EKG) was used to monitor heart patterns during the exercise stress test. To attain proper conductance and transmission of electrical potentials at the sites of electrode placement the skin was cleansed.

Skin was abraded with alcohol and gauze until red in order to remove skin oil and dead epithelial cells. When dry, tincture benzoine was applied to prepared sites as an adhesive and pre-gelled disposable electrodes (NDM, Braintree, Massachusetts) were placed on the following sites in the order described:

1. Limb leads (Diagram in Appendices)
    a. Right and Left Arm leads were located on the midclavicular line just below the clavicle.
    b. Right and Left Leg leads were located on the anterrior surface of the abdomen, just above the illiac crests, and on a line running between the navel and the anterior superior iliac spine.
2. Chest leads (Diagram in Appendices)
    a. V1 and V2 are over the fourth intercostal space, V1 on the right margin of the sternum and V2 on the left.
    b. V4 is placed on the fifth intercostal space along the midclavicular line.
    c. V3 is located in the middle of an oblique line connecting V2 and V4.
    d. V6 and V5 are placed on a horizontal line from V4 to the midaxillary line. V6 is located at the intersection of the horizontal and midaxillary lines, and V5 is halfway between V4 and V6.

c. Treadmill Test

Once electrodes were attached, the subject was seated next to the treadmill in a blood drawing chair. A light weight, adjustable plastic helmet which holds the mouth piece was placed on the subject's head, a rubber mouthpiece was inserted, and the subject's nose was plugged. The mouthpiece was attached to a three way valve which allowed the subject to breath room air and collected all expired gas through plastic tubing connected to the mouthpiece. This type of system is referred to as open circuit spirometry.

Expired gas was analysed by the Beckman MMC Metabolic Cart (Beckman Instruments, Schiller Park, Ill.) which contains an OM-11 polarimetric oxygen analyzer and an LB-2 infrared carbon dioxide analyzer. The metabolic cart was warmed up for three hours prior to calibration according to the Beckman MMC manual. A full calibration was done each morning before the cart was used and a quick calibration was done between each subject using two standard gas mixtures. The cart contains a calculator which was preprogrammed with the subject's weight. Measurements including oxygen consumption, tidal volume, respiratory rate, and respiratory quotient were averaged each thirty seconds and printed on a display tape.

Subjects remained seated until a true baseline oxygen consumption could be measured. This was a period of five to fifteen minutes. Baseline was taken as the point at which the respiratory quotient was below Ø.8Ø and had not decreased during the three previous measurements.

Subjects performed a maximum EKG treadmill test according

to the Bruce protocol (American Heart Association, 1972). This is a continuous multistage treadmill test consisting of seven stages. Electrocardiogram and blood pressure were measured two minutes into each stage. At the end of each three minute stage the treadmill increased in both speed and grade. A subject continued to exercise until she determined that she could no longer perform. Both the physician and investigator were informed about the contraindications for continuing an exercise test but no test had to be terminated before exhaustion. The Bruce Protocol and the American College of Sportsmedicine "Contraindications for continuing an Exercise Test" (American College of Sports Medicine, 1975) are found in the appendix.

d. Recovery and Post-Exercise Blood Sample

Immediately upon stopping the treadmill, the subject was helped into a supine position on the blood drawing chair. An immediate recovery EKG and blood pressure were taken. Expired gas was collected for twelve minutes post-exercise and an EKG was taken every three minutes during the recovery period.

At one minute post-exercise a 20 milliliter (ml) blood sample was drawn through the antecubital vein. Post-exercise hematocrit was measured and post-exercise blood samples were later analysed for E2, P, T, C and B-endorphin.

e. Body Fat Determination

Percentage of body fat was determined both by the skinfold technique and by hydrostatic weighing. Body fat content was estimated by the skinfold technique by measuring a double thickness of subcutaneous fat at seven anatomical sites using Lange skinfold calipers. Measurements at each location were repeated until two successive readings were within one millimeter of each other. The thumb and forefingers pinched the subcutaneous fat and calipers were placed beneath the fingers. All measurements were taken on the right side of the body at the specific anatomical sites described below and illustrated in the appendix.

1. Chest-midway between the nipple and axillary fold on a slant.
2 Subscapular-a diagonal measurement just below the inferrior border of the scapula.
3 Triceps-vertical measurement midway between the shoulder and elbow.
4. Abdomen-horizontal measurement just above the illiac crest.
5. Front Thigh-vertical pinch midway between knee and illiac crest. Subject bears body weight on left leg for this measurement.
6. Suprailliac-a diagonal measurement just above and parallel to the illiac crest.
7. Axilla-vertical fold at the middle of the side; on a line bisecting the armpit and hip and on the level

of the xiphoid process.

Percentage of body fat by the skinfold technique was calculated using the equation of Pollock et al., 1978 found in the appendix.

Hydrostatic weighing was performed on a subject seated in a chair which is suspended from a scale over a large tank of water. A subject made a forced maximal exhalation which caused her to be completely submerged and her weight underwater was read on a scale. Five or eight measurements were made and the median value was used to determine body fat according to the following equation of Katch and McArdle, 1977.

a. Underwater Weight=Scale Reading - Apparatus Weight

b. Body Density= $\frac{\text{Weight in Air}}{\text{Weight in Air} - \text{Weight in Water}}$ -Residual Water Temperature Correction Volume

c. Percent Fat= $\frac{495}{\text{Density}} - 450$

The residual volume measurement necessary for this equation was measured by helium dilution spirometry using a Gould (Pulmonet 3) spirometer according to the methods of West, 1979. The spirometer was calibrated before each use according to the Gould manual.

2. Part II: The Effect of Chronic Exercise on Resting Gonadotropin Pulsatility

Both runners and controls participated in this part of the study during the early follicular phase of their menstrual

cycles. They were instructed to refrain from ingesting caffeine, exercising, and taking aspirin for the twelve hours before their participation. All tests began at 0800 at which time an indwelling angiocatheter was inserted into a forearm vein and kept patent with 1 ml of 0.1% sodium heparin solution in saline.

At 0830 a 10 ml baseline blood sample was drawn for analysis of LH and FSH. Baseline E2 and P were also measured to verify the phase of the menstrual cycle. Following this, 2 ml of blood were drawn every 15 minutes for 6 hours for the analysis of the pulsatile patterns of LH and FSH. Before each blood draw a 1 ml syringe was used to clear the catheter of heparin.

Subjects were allowed to read or watch any of three non-provocative video movies provided by the investigator. Subjects brought their own lunches which they ate at 1200 hours and which contained no caffeinated beverages or chocolate. They were not allowed to sleep during the test or to walk anywhere except to the restroom.

3. Part III: The effect of Acute Exercise on Gonadotropin Pulsatility

Approximately one month later during the early follicular phase of their menstrual cycles, eumenorrheic runners returned to the laboratory. Again they had refrained from ingesting caffeine or exercising for twelve hours before the

study. A subject's dry land weight was measured and electrodes for a twelve lead electrocardiogram were applied as described in Part I. Then a subject was seated in a blood drawing chair and allowed to sit quietly for ten minutes before a 30 ml resting blood sample was drawn from the antecubital vein. This baseline sample was later analysed for E2, P LH, FSH, C, and B-endorphin.

Next, each runner performed a one hour run at 75 to 80 percent of her maximum oxygen consumption as determined in Part I. This was a treadmill run on a three degree incline, done to simulate a normal training run. During the first five minutes of the run the treadmill was increased until normal training pace was reached and the subject's heart rate was within + 2 beats per minute of her heart rate at 75 percent of her maximum oxygen consumption during the initial treadmill test. In some cases the speed of the treadmill was decreased slightly during the second half hour due to the runner's fatigue.

Heart rate and elecrocardiogram were monitored continuously during the run and a twelve lead electrocardiogram was taken every ten minutes. Oxygen consumption was measured during minutes 25 to 27 of the run. At minute 24 of the run a nose clip was placed on the subject's nose and a mouthpiece inserted. Expired gas was collected and analyzed by the Beckman MMC metabolic cart for six thirty-second periods. The subject then removed her mouthpiece. Running continued throughout the oxygen consumption measurement. At the end of one hour the

treadmill was stopped and the subject was helped to a supine position in the blood drawing chair. An indwelling angiocatheter was inserted into a subject's forearm vein and a 30 ml post-exercise blood sample was drawn for analysis of E2, P, LH, FSH, C and B-endorphin. Following this, blood samples were drawn every fifteen minutes for six hours according to the procedures described in Part II. The fifteen minute samples were used to assess levels and pulsatility patterns of LH and FSH. Also cortisol was measured on samples taken at 60, 120, 180, 240, and 360 minutes after the one hour run.

C. METHODS OF ANALYSIS

1. Sample Preparation

All blood was collected in tubes containing liquid ethyldiamintriacetic acid (0.5 ml of 10% EDTA), mixed thoroughly, and stored in ice before centrifugation. Samples were spun in a centrifuge (Sorvall RC3B, DuPont Co., Wilmington, DE) refrigerated to 4 degrees celsius for thirty minutes at 3000 revolutions per minute. Plasma was separated from red cells and stored at -30 degrees Celsius.

Plasma samples were thawed in a refrigerator overnight before use. After thawing, they were kept on ice at all times. Samples were mixed before use. In some cases it was necessary to spin the samples at 2500 revolutions per minute for fifteen minutes to separate the plasma from some fibrin clots. Clots were removed using a wooden stick coated with

prothrombin.

2. Gonadotropin Analysis

a. Iodination of the Hormones

1. Preparation of Sephadex G-75 Gel Column

A 25 ml glass pipet was placed on a ring stand. A small glass wool plug was inserted into the tip and a one inch plastic tube was attached to the tip of the pipet and closed with a small binder clip. The pipet was filled halfway with Ø.1% phosphate buffered saline gel (PBS-G) at pH 7.1 at room temperature (instructions in appendix).

A fifty percent slurry was made using Sephadex G-75 (Pharmacia, NJ) and PBS at pH 7.1. This solution was equilibrated at room temperature for 24 hours before use to allow the "gel to swel¹". The 25 ml pipet was then filled to the top with the fifty percent slurry. The column was opened and allowed to drain into a collection beaker while additional slurry was added to the column. This continued until the column was filled with the sephadex gel beads.

Next 1 ml of a five percent egg white solution in PBS plus 1 ml of transfer solution were combined. This solution was layered evenly on top of the gel bed and moved slowly down the column in a blue band coating the glass with egg white to prevent adherance of other protiens. PBS was added to the column until the band reached the bottom. Then the plastic tube was clipped shut and the column was ready for use.

2. Iodination of Hormones by the Chloramine-T Method

Iodination grade human luteinizing hormone, NIADDK-hLHI3, was obtained from the National Hormone and Pitutary Agency in Baltimore, MD. Under an iodination hood 2Ø micrograms of hLH in Ø.Ø25 mls of distilled water were added to Ø.Ø25 ml of Ø.5M Phosphate buffer (pH 7.6) and mixed. Next one millicurie of radiolabelled iodine 125 obtained from Amersham (Arlington Heights, IL) and Ø.Ø5 mls of Chloramine T (2 mg/ml solution with Ø.Ø5M phosphate buffer pH 7.5) were added to the hormone and agitated vigorously for two minutes. Chloramine T allows the attachment of labelled iodine to the tyrosine groups of the hormone by a redox reaction. The reaction was stopped by adding 5Ø micrograms of sodium-meta-bisulfite (2.5 microgram per milliliter solution with Ø.Ø5M phosphate buffer, pH 7.6). Then the iodination reaction mixture was layered onto the top of the column and the plastic tube on the end of the column was unclipped allowing it to flow at a rate of about 1 ml per minute. One minute fractions of eluent were collected for fifteen minutes in tubes containing 1 ml of Ø.1% PBSG. A 1Ø microliter fraction of each tube was counted to determine which fractions contained the bound hormone. These fractions were combined and reserved for use in the assay

The procedure for the iodination of hFSH was very similar to that of hLH. Iodination grade hormone, NIADDK-hFSH-3, was obtained from the National Hormone and Pitutary Agency. Five micrograms of this hormone in Ø.Ø4Ø ml of distilled water

were used for each iodination. The iodination for hFSH from this step on was identical to that described for hLH except that only Ø.Ø3 ml of sodium meta-bi-sulfite (2 microgram per ml solution with Ø.Ø5M phosphate buffer, pH 7.6) was used.

3. Preparation of Chloride Ion Exchange Columns

Depending on availability, in some cases hormones were iodinated using a chloride ion exchange column. A 3 ml plastic syringe was attached to a ring stand and a glass wool plug was placed inside its port. A one inch plastic tube was placed on the port and fastened shut with a binder clip. A fifty percent slurry was made using BioRad Analytical Grade Anion Exchange Resin AG 1-xlØ, 2ØØ-4ØØ mesh chloride form (BioRad, Richmond, CA) and Ø.Ø5M phosphate buffer pH 7.6 at room temperature.

First the column was filled with 3 mls of the slurry. The clip was then opened and the buffer allowed to drain into a collection cup. Next 3 mls of Ø.Ø5M phosphate buffer pH 7.6 were layered onto the top of the column followed by 2 mls of Ø.5M phosphate buffer pH 7.6. Then 2 mls of Ø.Ø5M phosphate buffer with bovine serum albumin were layered onto the column and the fluid was allowed to drain to the top of the resin bed. Finally 2 mls of Ø.Ø5M phosphate buffer were added and drained so it just covered the top of the resin.

4. Iodination of Hormones and Purification Using the

Chloride Ion Exchange Method

The iodination reaction mixtures for hLH and hFSH are prepared in the same manner as described for the G-75 Sephadex columns. The reaction mixture was layered evenly on top of the resin bed. The column was then opened and the reaction mixture entered the bed. Two mls of Ø.Ø5M phosphate buffer were added to the top of the column to elute off the hormone. All of the eluent was collected in one tube containing 1 ml of Ø.1% PBSG.

b. Antibody Titer and Pilot Studies

The antibodies used in this study were NIADDK-anti-hFSH-6 and antiserum to hLH batch #2 (Rabbit anti-human LH provided by the National Hormone and Pitutary Program. Classification studies provided with the antibodies show that there was less than one percent cross reactivity with other pituitary hormones.

Antibody titer studies were performed according to the gonadotropin assay procedures described in the next section and using serial dilutions of antibody. The antibody dilutions of NIADDK-anti-hFSH-6 ranged from 1:1Ø,ØØØ to 1:24Ø,ØØØ. These studies showed that maximum binding of 22.6% occured at an antibody dilution of 1:1Ø,ØØØ and that binding fell to 14.2% at a 1:2Ø,ØØØ antibody dilution. Antibody titer studies of hLH antibody Batch #2 revealed that optimal binding of 24.2% occured at antibody dilutions ranging from 1:4Ø,ØØØ to 1:6Ø,ØØØ. Binding fell to 1Ø.3% at

an antibody dilution of 1:240,000.

c. Radioimmunoassay for Human Luteinizing Hormone

Plasma luteinizing hormone levels were determined by a double antibody radioimmunoassay according to the procedures provided by the National Hormone and Pituitary program. Standard solutions were prepared from reference material LER907 and used in solutions of 0.062 mIU/0.05 ml to 32.00 mIU/0.05 ml in 0.1% PBS-G pH 7.2 to construct a standard curve. The minimal detectable level of hLH in this system was approximately 0.125 mIU/ml. Normal rabbit serum (NRS) diluted 400-fold with 0.05M EDTA PBS was used to determine the degree of non-specific binding.

The assay was done over a four day period and incubated at 4 degrees Celsius between the addition of reagents. The first day 0.1 ml of an unknown serum sample was added to 0.4 ml of assay buffer, 0.1% PBSG pH 7.2. Also 0.2 ml of hLH antibody at a concentration of 1:140,000 in NRS diluted 1:400 in 0.5M EDTA PBS was added. After a 24 hour incubation 0.1 ml (approximately 25,000 counts per minute) of labelled hLH diluted in 0.1% PBSG was added. On day three 0.2 ml of second antibody, sheep antirabbit gamma globulin (SARG pool C) in a 1:60 dilution with PBS was added. After each addition the test tubes were vortexed. All samples were assayed in duplicate.

Following a 72 hour incubation period, each tube had 1 ml of PBS added and was centrifuged at 3000 revolutions per minute for 30 minutes. The supernatant was discarded and

tubes were allowed to drain for 15 minutes. The remaining pellet was counted in a gamma scintillation counter (Gamma Trac, Tm Analytical, Millford, MA.). When a sample's LH values fell below the mean detectable level it was reassayed using Ø.2 mls of serum and Ø.3 mls of assay buffer. Duplicates of Ø.Ø25 ml, Ø.Ø5 ml, and Ø.1Ø ml of the same control sample were run in each assay to determine interassay coefficient of variation which was 11.Ø%. Intraassay coefficient of variation as determined by running ten Ø.1 ml control samples in a single assay was 6.Ø%.

d. Radioimmunoassay for Human Follicle Stimulating Hormone

Plasma follicle stimulating hormone levels were also determined by a double antibody radioimmunoassay. The methods were modified from the guidelines of the National Hormone and Pituitary Agency to increase the assay sensitivity. The standard solutions were prepared from reference material LER9Ø7 and used in solutions from Ø.5 mIU/Ø.5 ml to 64mIU/Ø.5ml diluted in Ø.1% PBSG pH 7.2 to construct a standard curve. The minimal detectable level of hFSH was approximately 1.Ø mIU/ml.

The assay was performed over a three day period and incubated at room temperature (25 degrees Celsius) between the addition of reagents. Either Ø.1 ml or Ø.2 ml of unknown sample were added to assay buffer, Ø.1% PBSG pH 7.2, to make a total volume of Ø.5 mls. To this Ø.2 mls of hFSH antibody (NIADDK-anti-hFSH-6) diluted 1:25,ØØØ with 1:4ØØ NRS in Ø.Ø5M

EDTA PBS was used. The 1:400 NRS without the hFSH antibody was used to determine non-specific binding.

After a 24 hour incubation period, 0.2 ml of second antibody (SARG Pool C) diluted 1:60 in PBS were added. One day later 1 ml of PBS was added to each tube and all tubes were centrifuged at 3000 revolutions per minute for 30 minutes. The supernatant was decanted from the pellet and the tubes were allowed to drain for 15 minutes.The pellet was counted in a gamma scintillation counter.

All unknown samples were assayed in duplicate. Tubes were mixed after the addition of each reagent. Plasma from a post menopausal woman was run in each assay as a control and to determine the interassay coefficient of variation which was 3.0%. The intraassay coeffient of variation, which was determined by assaying ten 0.1 ml samples of this control in a single assay, was 5%.

3. Steroid Hormone Analysis

a. Steroid Extraction

One ml of plasma and 5 mls of diethyl ether were aliquoted into a scintillation vial (Wheaton Scientific, Millville, NJ) and vortexed for 30 seconds. The samples were snap frozen in liquid nitrogen and the steroid containing organic layer was decanted into another scintillation vial. The samples were evaporated (Multivap Organomation Associated Inc., Shrewsbury, MA) and reconstituted in 1 ml of ethanol.

Prior to extraction 0.1 ml of the appropriate

radiolabelled hormone (1000 to 1200 counts per minute) was added to the sample. Then 0.1 ml of reconstituted sample was pipeted into a biovial and counted on a beta scintillation counter (Mark III, Model 6882, Tracor Analytic, Elk Grove Village Il). Percent recovery was calculated as the percentage of total counts added.

b. Estradiol

Estradiol was quantitated by radioimmunoassay according to the methods of Korenman (1974). Standard solutions diluted with ethanol ranged from concentrations of 0 pg per tube to 200 pg per tube. The minimum detectable value was 3 pg of E2 per tube.

Next, 0.25 ml of reconstituted unknown sample in duplicate was air evaporated. The radiolabelled hormone (2,4,6,7 3H)-estradiol, specific activity 85-110 Ci/mMol, Amersham, Arlington Heights, IL) was utilized for this assay and 0.1 ml was added to each tube (approximately 10,000 cpm). Also 0.1 ml of estradiol antibody #244 diluted to 1:40,000 with PBSG was added to standard and unknown tubes and the assay was incubated for 24 hours at 4 degrees Celsius.

After incubation 0.5 ml of dextran-charcoal suspension (refer to appendix) was added to each tube, vortexed, and the assay was incubated on ice for 10 minutes. The tubes were centrifuged at 3000 revolutions per minute for 10 minutes and the supernatant was decanted into biovials. Two and one half mls of scintillation cocktail (Hydroflour, National Diagnostics, Sommerville, NJ) were added, mixed, and the

biovials were counted in a beta counter. Estradiol levels were measured in two assays. Interassay coefficient of variation was 8.0% and intraassay coefficient of variation was 10.0%.

c. Progesterone and Testosterone

Progesterone was measured according to the methods of Koligan (1977) and testosterone was quantitated following the procedures of Chakraborty (1984). Standards for progesterone and testosterone ranged from 0.01 ng per tube to 1.00 ng per tube. Reconstituted unknown (0.1 ml) was pipeted in duplicate and unknowns and standards were air evaporated in scintillation vials.

Each scintillation vial received 0.1 ml of isotope labelled hormone, either (1,2,5,7, 3H)-progesterone, specific activity 80-110 Ci/mMol, Amersham) or (1,2,6,7 3H-testosterone, specific activity 85-105 Ci/mMol, Amersham), which had 10,000 to 12,000 counts per 0.1 ml. The appropriate antibody (0.1 ml) was added to all standards and unknowns. The progesterone antibody (#377) was used at a dilution of 1:2000 and the testosterone antibody (#S250) was used at a dilution of 1:50,000 in 0.1% PBSG. The testosterone antibody has a cross-reactivity with dihydrotestosterone (DHT) of approximately 74 percent. Therefore, it was incubated with 4 ng per ml DHT for 48 hours prior to use which reduced its cross-reactivity to 10 to 15 percent. Tubes were vortexed after each addition and were

incubated for 2 hours at room temperature after the addition of antibody.

After incubation, tubes were placed in an ice bath for 10 minutes. Then 0.5 ml of Dextran-Charcoal in PBSG suspension was added and tubes were vortexed and incubated on ice for 10 minutes. Finally tubes were centrifuged at 4 degrees Celsius for 10 minutes at 3000 revolutions per minute. The supernatant was decanted into biovials and 2.5 mls of scintillation cocktail were added. Each sample was counted for one minute in a beta counter. The interassay coefficient of variation was determined for all steroids by running two control samples of the appropriate hormone in each assay was 8.0% for progesterone. All testosterone measurements were done in one assay. Ten control samples were run in a single assay for determination of intraassay coefficient of variation which was 11.0% for progesterone and 16.0% for testosterone.

d. Cortisol Analysis

Determination of cortisol values was made utilizing a RAINEN Cortisol (I125) Radioimmunoassay kit (New England Nuclear, North Billerica, MA) and was performed according to the instructions provided. Standards for this assay ranged from 2 to 50 micrograms percent. Also two control samples of 4 and 20 micrograms percent were run in this assay.

To 0.01 ml of unknown plasma sample were added 0.5 ml of (I125) labelled cortisol tracer and 0.5 ml of cortisol

antiserum complex, a mixture of first and second antibody containing rabbit antibody to cortisol prereacted with an antiserum to rabbit gamma globulin. This mixture was vortexed and incubated at room temperature for 30 minutes. After incubation tubes were centrifuged at 4 degrees Celsius for 10 minutes at 3000 revolutions per minute. The supernatant was decanted and the pellets were each counted for 1 minute in a gamma counter. All samples were measured in one assay and intraassay coefficient of variation was 2.9%.

e.Beta-Endorphin Analysis

1. Extraction of Beta-Endorphin

Samples were chromatographed on a Sep Pak C18 cartridge (Waters Associated Inc., Milford, MA) which is a reverse phase liquid chromatographic system which retains hydrophobic species. These were placed in a Sep-Pak Rack(Waters Associates Inc., Milford, MA) and separation was aided by vaccuum suction. The column was activated with 5 mls of a 0.05% trifluoracetic acid (TFA) solution in 100% acetonitrile and then initialized with a 0.05% TFA solution in distilled water. Activation and initialization were at a rate of 1 drop each 2 seconds as controlled by a vaccuum suction outlet.

Four to 6 mls of unknown plasma sample were layered onto the column. The columns were washed with 3 mls of 0.05% TFA in distilled water to remove the polar constituents in the

plasma. Then B-endorphin was eluted off the column using 3 mls of Ø.Ø5% TFA in a 75% acetonitrile solution diluted with distilled water. Layering, washing, and eluting were at a rate of one drop each 1Ø seconds.

Samples were spun in a Speed Vac Concentrator (Savant Instruments, Hicksville, NY) for one hour to remove the organic solvents then frozen completely at -7Ø degrees Celsius. After freezing, samples were lyophilized (Freezemobile 6; Virtis Co. Inc., Gardener, NY) for 24 hours and stored in air tight containers until they were assayed.

2.Beta-endorphin Radioimmunoassay

Prior to layering, all samples and controls were spiked with (I-125) acetylated B-endorphin 1-27 (Ac B-End-1-27) at 1ØØØ counts per minute for use as an internal standard. Lyophilized samples were resuspended in 1.Ø ml of a mixture of 5 parts assay buffer, 1 part heat inactivated horse serum (HIHS) and 2 parts antibody as described below, and were counted for recovery of internal standards on a gamma scintillation counter before performing the assay.

Heat inactivated horse serum (Ø.5 ml) in a 1:4 solution with assay buffer was added to each tube prior to addition of standard or sample in order to protein coat the glass tubes. A phosphate assay buffer (refer to appendix) plus antibody (C-55 rabbit anti-camel B-endorphin) at a dilution of 1:14,ØØØ with assay buffer were also in the resuspension mixture. This antibody is directed against the 17 to 25

amino acid portion of B-endorphin and therefore recognized both Beta-lipotropin and B-endorphin on an equimolar basis. Duplicate aliquots of Ø.4 ml, which each contained unknown, Ø.1 ml antibody, Ø.Ø5 ml of HIHS, and Ø.25 ml of buffer, were added to each assay tube. Resuspended samples were assayed undiluted, and at 1:5, and 1:25 dilutions. B-endorphin 1-27 (Ø.1 ml), -N-acetylated and radiolabelled with I-125, were added to each assay tube. Tubes were then incubated at 4 degrees Celsius for 48 hours. Afterwards 1 ml of 18% polyethelene glycol in distilled water was added to each tube and they were centrigfuged at 4 degrees Celsius for 6Ø minutes at 25ØØ revolutions per minute. Supernatant was aspirated and the pellets were each counted for one minute on a gamma counter.

Data were expressed as B-endorphin/B-LPH immunoreactivity and quantitated using standards of camel Ac-B-endorphin 1-27 (Peninsula Laboratories, Inc., Belmont, CA). ranging from 1 femtomole to 3ØØ femtomoles and all samples were run in a single assay. A control sample containing a known quantity of Ac-B-endorphin 1-27 was run with each Sep-Pak rack to determine intraassay variability which was 1Ø.6%. The minimal detectable level for this assay was 5 femtomoles. Recovery of B-endorphin by immunoreactivity averaged 77.6%.

f.Analysis of Data

Standards were calculated by averaging the values of duplicates and dividing this number by the zero standard to

determine percent binding. Then a calculator (Canon CP2020, Alexandria, VA) preprogrammed to perform linear regression was utilized to determine the concentration of the unknown by the four parameter logistic curve fitting program (Grotjan, 1977). The average value for the assay's non-specific binding was subtracted from standard and sample values prior to calculation. The concentrations were multiplied by their appropriate dilution factors. Differences between means were tested for statistical significance by application of Student's t-test (Steel, 1960) with a type I error of 0.05. Unpaired t-tests were used to test significance between runners and non-runners while paired t-tests were used to compare pre and post exercise values within a single group. When outliers were present, statistical difference was also determined by the Wilcoxin Rank-Sum test (Downe, 1974).

IV. RESULTS

A. DESCRIPTION OF STUDY POPULATION

Twenty-four women originally voluteered to participate in this study. However, due to scheduling problems, injuries, and one pregnancy, only nineteen women completed all portions of the study. Unless otherwise specified, the following section reports on data collected from nine normally menstruating long distance runners and nine eumenorrheic sedentary controls.

Runners and non-runners were well matched in age (32.5±1.6 vs. 31.5±1.9 years mean±SEM) body weight (56.3±1.5 vs. 60.0±2.1 kg.) and height (165.0±1.66 vs. 164.6±1.64 cm.) as shown in table 3. Runners had significantly lower percentages of body fat as compared to non-runners both by the hydrostatic (16.4±7.5% vs. 24.4±2.3% $p<0.01$) and skinfold methods (13.5±1.1% (N=2) vs. 25.2±1.4% (N=8) $p<0.05$). Also, as would be expected with aerobic training, runners had significantly lower resting heart rates than non-runners (60.1±5.2 vs. 73.9±3.1 beats/minute $p<0.05$, table 4).

Runners and non-runners did not differ significantly as to age at menarche (12.93±0.25 vs. 11.38±1.28 years) or the length of their menstrual cycles (29.43±2.24 vs. 26.86±0.83 days) and all cycle lengths were within the normal reported

Table 3 Runners and controls were well-matched in age, weight, and height. Runners had significantly lower body fat and a greater parity than non-runners.

Table 3

DESCRIPTION OF SUBJECT POPULATION

	MEAN±SEM	NON-RUNNERS (n=10)	RUNNERS (n=9	SIGNIFICANCE
1.	Age (years)	31.5±1.9	32.5±1.6	N.S.
2.	Weight (kg)	60.0±2.1	56.3±1.5	N.S.
3.	Height (cm)	164.6±1.6	165.0±1.7	N.S.
4.	% Fat			
	Hydrostatic Weighing	24.4±2.3	16.4±7.5	$p<0.01$
	Skinfold	25.2±1.4	13.5±1.1	$p<0.05$
5.	Age at Menarche (years)	11.4±1.3	12.9±0.3	N.S.
6.	Length of Menstrual Cycle (days)	26.9±0.8	29.4±2.2	N.S.
7.	Parity (#having at least 1 child)	1	5	-
8.	Number of years of training	-	8.1±1.0	-
9.	# miles run per week (mi/wk)	-	50.9±1.9	-

range of 24 to 36 days (Wilson, 1985). Five of the runners had had at least one pregnancy while only one of the non-runners had ever been pregnant. None of the control subjects had ever had menstrual irregularities aside from dysmenorrhea. Two of the runners had once been amenorrheic but both had been eumenorrheic for at least six years prior to the study and one had given birth in the interum.

The runners in this study had been training for an average of 8.13±1.09 years (range 3 to 12 years) and average 50.9±1.9 miles per week of running for the three months prior to the study. All of these women were competetive runners racing frequently and all but one had completed a marathon within the past three years. Two of the women were nationally ranked runners and one had participated in the Olympic Marathon Trials.

B. RESPONSES TO ACUTE EXERCISE

1. Maximum EKG Treadmill Test

All subjects performed a maximum EKG treadmill test according to the Bruce Protocol. Heart rate and EKG were monitored before, during, and after the exercise test. Blood samples were taken before and immediately after completion of exercise. This portion of the study was performed to verify the fitness levels of the two study populations and to investigate the humoral responses to acute maximum exercise in trained and untrained women.

As shown in table 4, runners had significantly higher maximum oxygen consumptions (45.Ø±1.3 vs. 28.7±1.4 ml/kg/min; p<Ø.Ø1) and exercised significantly longer on the treadmill (14.7±Ø.6 vs. 1Ø.3±1.2 mins.; p<Ø.Ø1) than non-runners. Maximum heart rates were somewhat lower in runners (178.Ø±3.2 beats per min) than controls (187.4±4.3 beats per min) but this difference was not significant. Both groups were exercising anaerobically at exhaustion as indicated by respiratory exchange ratios that were well over 1.ØØ. In fact, sedentary controls had significantly higher respiratory exchange ratios at peak exercise than did runners (1.3Ø±Ø.Ø3 vs. 1.19±Ø.Ø8; p<Ø.Ø1).

2. Humoral Responses

There were no significant changes in serum hematocrit as a result of treadmill exercise (44.6 vs. 45.Ø, S.E. Ø.6, runners; 43.8 vs. 45.7, S.E. Ø.2, non-runners; pre- vs. post-exercise) as shown in table 5. Nevertheless, individual post-exercise values were corrected for changes in hematocrit by the method of Van Beaumont (1973). This technique is illustrated in the appendix. Presently there is an argument among exercise physiologists as to whether post-exercise values should be corrected for changes in hematocrit. Some argue that exercise-induced changes in a particular serum constituent should be distinguished from those changes caused by hemoconcentration. However, others believe that it is the post-exercise concentration of the

Table 4 Verification of the runners' high training level was seen by significantly higher maximum oxygen consumptions, longer times on the treadmill, and lower resting heart rates. Comparisons between groups were calculated by unpaired t-tests.

Table 4

COMPARISON OF THE RESULTS FROM THE MAXIMUM TREADMILL TEST ON RUNNERS AND CONTROLS

	MEAN±SEM	NON-RUNNERS (n=10)	RUNNERS (n=9)	SIGNIFICANCE
1.	Maximum Oxygen Consumption (ml/kg/min)	28.7±1.4	45.0±1.3	$p<0.01$
2.	Time on Treadmill (mins)	10.3±1.2	14.7±0.6	$p<0.01$
3.	Resting Heart Rate (beats/min)	73.9±3.1	60.1±5.2	$p<0.05$
4.	Maximum Heart Rate (beats/min)	187.4±4.3	178.0±3.2	N.S.
5.	Respiratory Exchange Ratio at Peak Exercise	1.3±0.0	1.2±0.1	$p<0.01$

Table 5 Hematocrit did not change significantly after maximal or submaximal exercise. Statistics were calculated within groups using paired t-tests.

Table 5

CHANGES IN HEMATOCRIT WITH EXERCISE

Subjects	Pre Treadmill	Post Treadmill	Difference (Post-Pre)	Standard Error	Pre 1 Hr.	Post 1 Hr.	Difference (Post-Pre)	Standard Error
Runners	44.6	45.0	0.4	0.6	41.4	39.9	-1.5	1.4
Controls	43.8	45.7	0.9	0.2				

*There were no significant differences in hematocrit between groups or as a result of exercise.

constituent which stimulates the response by the end organ and that it does not matter whether this concentration was attained by a change in its production rate or by hemoconcentration. In any case, both the actual measured post-exercise value as well as the post-exercise value corrected for changes in hematocrit are presented for each hormone measured.

There were no significant differences in pre-treadmill steroid levels as shown in table 6. This is as would be expected due to the variability of steroid hormones measured at random times in the menstrual cycle. However, based on the data from menstrual questionnaires which included date of last cycle and normal cycle length, four runners and three non-runners were in the luteal phase of their cycles. Runners had lower luteal phase progesterones than non-runners (1.2±Ø.3 vs. 3.3±Ø.7 ng/m^1) and this difference approached but did not attain significance. There were no differences in any of the other steroid hormones when examinede according to phase of the cycle.

There were no significant changes in estradiol, progesterone or testosterone within groups as a result of maximum treadmill exercise. Estradiol decreased slightly after exercise in runners (21Ø±42 vs. 2Ø7±36 pg/ml pre vs. post) and to a greater extent in controls (28Ø±94 vs. 135±12 pg/ml pre vs. post). Progesterone levels changed very little after treadmill exercise (6.4±2.7 vs. 5.8 ±2.5 ng/ml runners; 2.2±Ø.8 vs. 2.3±Ø.9 ng/ml controls, pre- vs. post-exercise). In both groups serum testosterone levels increased slightly

Table 6 This table shows pre and post treadmill run steroid values in runners and controls. Post exercise values were corrected for exercise-induced changes in hematocrit and compared to pre-exercise values. Pre and post exercise comparisons within groups were done by paired t-tests. Comparisons between runners and controls were calculated by unpaired t-tests. Steroid levels did not change in runners or controls after maximal treadmill exercise.

Table 6

PRE AND POST TREADMILL STEROID LEVELS IN RUNNERS AND CONTROLS

	MEAN±SEM	PRE E2(pg/ml)	POST E2	POST E2 CORR HCT	PRE P(ng/ml)	POST P	POST P CORR HCT	PRE T (ng/ml)	POST T	POST T CORR Hct
1.	Runners	210.00 ±42.00	207.00 ±36.00	180.30 ±39.00	6.40 ±2.70	5.80 ±2.50	5.70 ±3.00	0.60 ±0.01	0.80 ±0.10	0.80 ±0.10
	% Δ from baseline		-0.06 ±0.08	-0.02 ±0.20		0.04 ±0.10	-0.13 ±0.10		0.56 ±0.28	75.00 ±75.00
2.	Non-Runners	280.00 ±94.00	135.00 ±12.00	107.00 ±9.00	2.20 ±0.80	2.30 ±0.90	1.40 ±0.60	0.50 ±0.10	0.70 ±0.10	0.60 ±0.10
	% Δ from baseline		-0.31 ±0.18	-0.53 ±0.17		-0.19 ±0.14	-0.24 ±0.07		0.06 ±0.08	0.07 ±0.11

*There were no differences in steroid levels between groups or within groups at rest or after exercise.

but not significantly (Ø.6±Ø.Ø1 vs. Ø.8±Ø.1 ng/ml runners; Ø.5±Ø.1 vs. Ø.7±Ø.1 ng/ml controls; pre- vs. post-exercise). In addition, the magnitude of the exercise-induced changes was not different between groups for any of the three steroid levels. Since treadmill tests were performed during random phases of the menstrual cycle, post-exercise steroid values are also expressed as the percent change from the pre-exercise value. Although this mathematical manipulation corrects for changes in steroid values during different phases of the menstrual cycle, there were still no significant exercise-induced changes in any of the steroids measured. These results are illustrated in table 6.

Maximum exercise did not evoke any significant changes in either LH or FSH as shown in table 7. Luteinizing hormone levels rose slightly post-exercise in non-runners (4.48±2.39 vs. 4.59±2.39 mIU/ml) and fell slightly in runners (8.4±2.Ø vs. 7.7±1.9 mIU/ml). Follicle stimulating hormone showed minor increases after exercise in both groups (6.Ø3±1.22 vs. 6.Ø8±Ø.91 mIU/ml non-runners; 7.47±1.11 vs. 7.67±1.62 runners). In addition, the exercise-induced rises in serum cortisol were slight and did not approach significance (1Ø6.Ø±18.8 vs. 111.4±15.2 ng/ml controls; 146.Ø±26.3 vs. 166.2±24.5 ng/ml runners pre vs. post exercise; table 8 and figure 5). However, there were dramatic increases in serum B-endorphin in both runners and controls (table 9 and figure 6). B-endorphin levels rose from 2.16±1.12 to 15.51±3.76 fm/ml in runners and from Ø.59±Ø.1Ø to 9.42±4.4Ø fm/ml in

Table 7 Gonadotropin levels did not change as a result of maximal or submaximal exercise. Treadmill tests were performed during random phases of the menstrual cycle. All runners did their one hour runs during the early follicular phase of their cycles.

Table 7

CHANGES IN GONADOTROPIN LEVELS IN RUNNERS AND CONTROLS AS A RESULT OF EXERCISE

	MEAN±SEM (mIU/ml)	LH PRE EX	LH POST EX	LH POST EX CORR HCT	FSH PRE EX	FSH POST EX	FSH POST EX CORR HCT
1.	Non-Runners Pre & Post Treadmill	4.48 ±2.39	4.59 ±2.39	5.80 ±3.20	6.03 ±1.22	6.08 ±0.91	6.60 ±5.70
2.	Runners Pre & Post Treadmill	8.40 ±2.00	7.70 ±1.90	6.08 ±1.80	7.47 ±1.11	7.67 ±1.62	7.60 ±1.90
3.	Runners Pre & Post 1 hr run	3.80 ±1.00	4.20 ±0.80	4.40 ±0.70	6.10 ±1.00	5.00 ±1.70	5.30 ±2.00

*There are no significant differences in gonadotropin levels either among groups or within groups as a result of exercise.

Table 8 Serum cortisol levels did not change after maximal or submaximal exercise.

Table 8

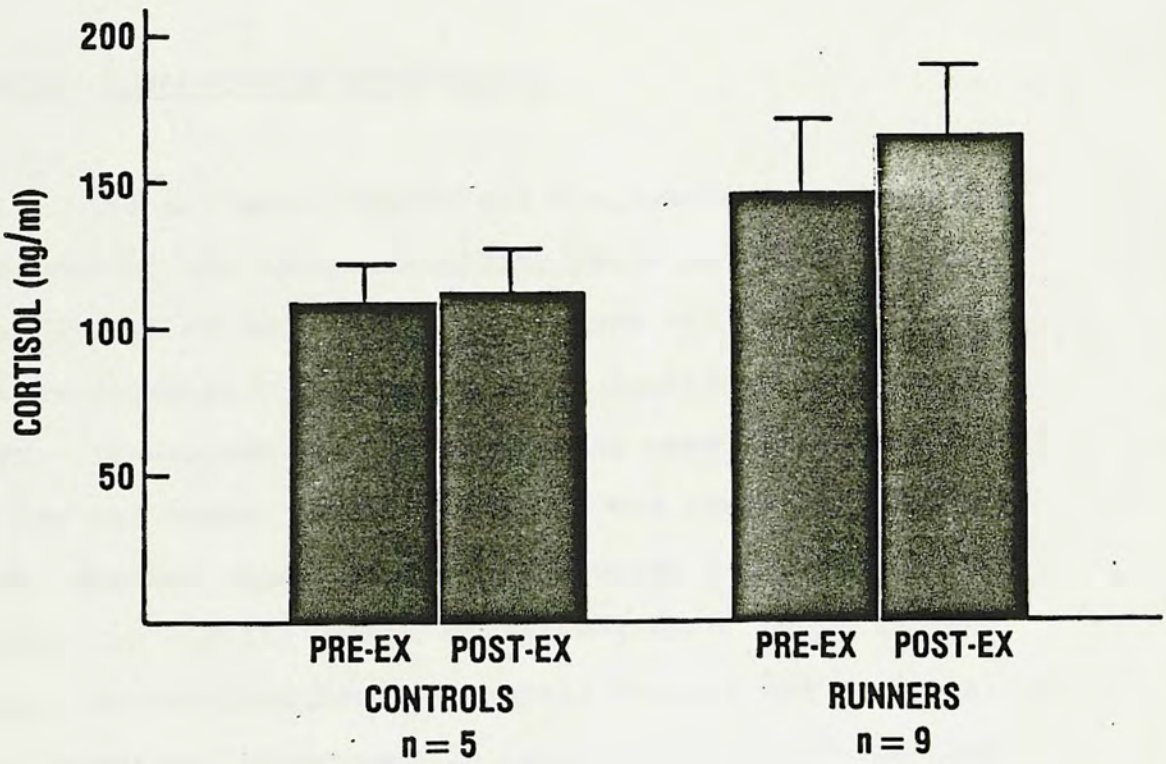
EFFECT OF EXERCISE ON SERUM CORTISOL LEVELS

	MEAN±SEM (ng/ml)	PRE EXERCISE	POST EXERCISE	POST EXERCISE CORR HCT	PRE 1 HR RUN	POST 1 HR RUN	POST 1 HR RUN CORR FOR HCT
1.	Non-Runners	106.0 ±18.8	111.4 ±15.2	104.8 ±15.9	- -	- -	- -
2.	Runners	146.0 ±26.3	166.2 ±24.5	166.8 ±27.5	91.8 ±22.1	66.0 ±20.1	69.4 ±26.4

*There were no significant differences in cortisol levels between groups or as a result of exercise.

Figure 5 There was no significant change in serum cortisol in either controls or runners as a result of maximal treadmill exercise.

THE RESPONSE OF SERUM CORTISOL TO MAXIMAL TREADMILL EXERCISE



non-runners. These changes were further accentuated when corrected for changes in post-exercise hematocrit. However, there were no significant differences in either resting or post-exercise B-endorphin levels between groups and no difference in the magnitude of the exercise-induced rise. In addition, there was a significant positive correlation between B-endorphin levels before and after exercise such that the higher one's resting B-endorphin level, the higher will be one's peak exercise level ($r=Ø.393$ $p<Ø.Ø5$).

C. RESTING GONADOTROPIN PULSATILITY

All subjects, both runners and non-runners, reported to the lab during the early follicular phase of their cycles (mean day $4.86\pm Ø.67$ vs. $4.8Ø\pm Ø.66$ runners vs. controls). An intravenous catheter was inserted and a baseline blood sample was drawn. Subsequently, blood samples were taken every 15 minutes for six hours. This portion of the study was done to determine whether there are any differences in gonadotropin pulsatility or resting levels of reproductive hormones, B-endorphin, or cortisol between trained runners and controls.

The baseline blood samples revealed that runners had significantly lower estradiol levels than controls ($68.Ø\pm 4.Ø$ vs. 88.6 ± 13.2 pg/ml; $p<Ø.Ø5$; figure 7 and table 1Ø) Testosterone levels were virtually identical between the two groups ($Ø.41\pm Ø.Ø6$ vs. $Ø.41\pm Ø.Ø4$ ng/ml; runners vs. controls) and progesterone levels were lower in non-runners than runners ($Ø.36\pm Ø.Ø6$ vs. $Ø.85\pm Ø.44$) and this difference

Table 9 Post exercise immunoreactive B-endorphin was significantly higher than pre-exercise values in both groups. Serum B-endorphin levels were not different between runners at rest or before and after maximal exercise. There was no change in B-endorphin after a normal training run in runners. Also, post-exercise values did not change significantly when corrected for exercise-induced alterations in serum hematocrit.

Table 9

BETA-ENDORPHIN IMMUNOREACTIVITY IN RUNNERS AND CONTROLS AT REST AND AFTER EXERCISE

MEAN±SEM (fm/ml)	PRE TREADMILL	POST TREADMILL	POST TREADMILL CORR. HCT.	REST EARLY FOLLICULAR	PRE 1 HR RUN	POST 1 HR RUN	POST 1 HR RUN CORR. HCT.
RUNNERS	2.16 ±1.12[a]	15.51 ± 3.76[b]	17.14 ±10.80[b]	1.85 ±0.46[a]	1.21 ±0.42[a]	1.32 ±0.70[a]	0.66 ±0.21[a]
CONTROLS	0.59 ±0.10[a]	9.42 ±4.40[b]	11.25 ±5.90[b]	1.83 ±0.90[a]	- -	- -	- -

*Figures within rows displaying different superscripts are significantly different $p<0.05$

Figure 6 Serum B-endorphin increased significantly after maximum exercise in both runners and controls. There were no differences between groups in B-endorphin response to exercise.

THE RESPONSE OF SERUM β-ENDORPHIN IMMUNOREACTIVITY TO MAXIMUM TREADMILL EXERCISE IN TRAINED RUNNERS AND SEDENTRARY CONTROLS

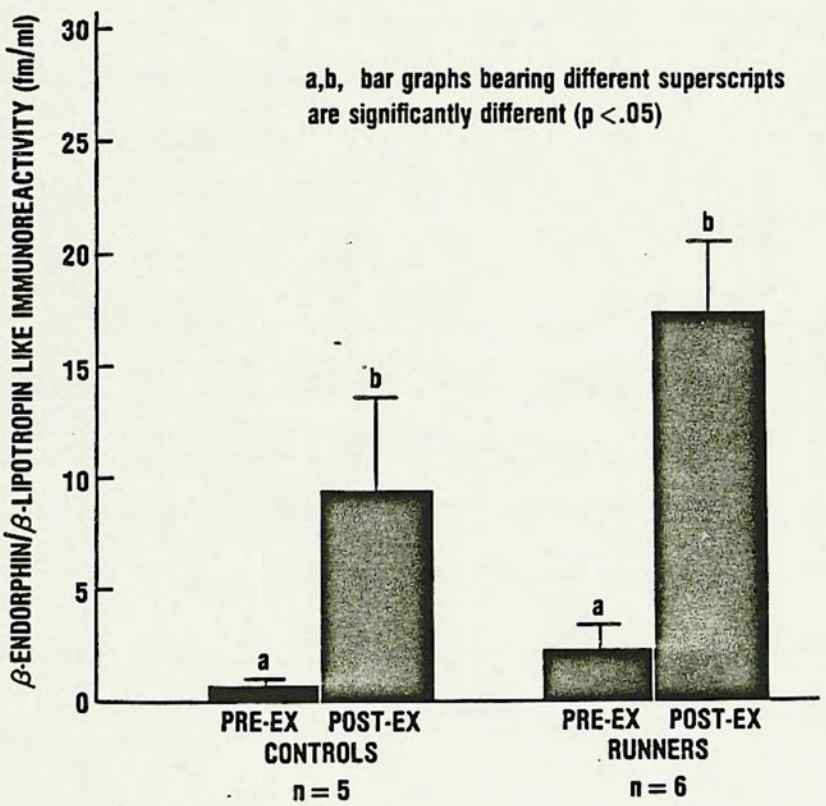


Figure 7 Serum estradiol levels were significantly lower in runners than controls. There were no differences in resting levels of progesterone or testosterone between groups. Significance for estradiol was determined by the Rank-Sum Test due to the presence of outliers. Significance of testosterone and progesterone levels were calculated by both unpaired t-tests and the Rank-Sum Test.

MEAN STEROID HORMONE CONCENTRATIONS OF RUNNERS AND NON-RUNNERS AT REST

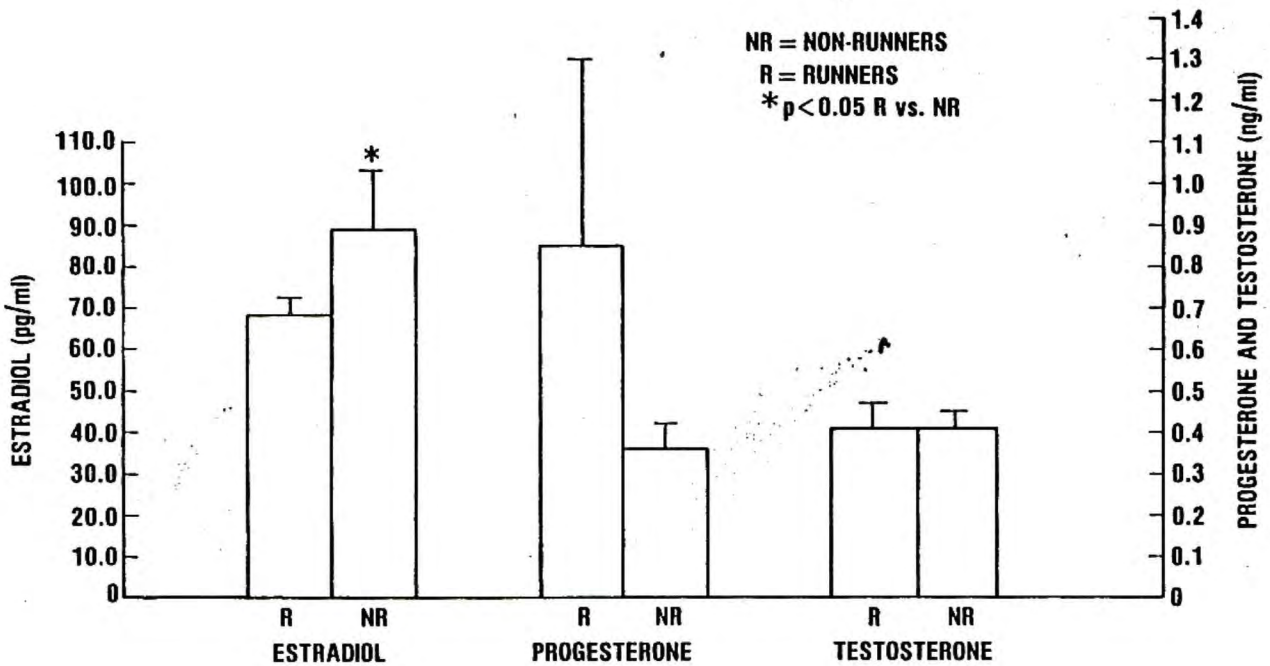


Table 1Ø Follicular phase serum estradiol levels were significantly lower in runners than controls. Progesterone and testosterone levels were not different between groups.

Table 10

EARLY FOLLICULAR PHASE STEROID HORMONE CONCENTRATIONS IN RUNNERS AND NON-RUNNERS AT REST

	MEAN±SEM	RUNNER	CONTROL	SIGNIFICANCE
1.	Estradiol (pg/ml)	68.0±4.0	88.6±13.2	$p<0.05$
2.	Progesterone (ng/ml)	0.85±0.44	0.36±0.06	N.S.
3.	Testosterone (ng/ml)	0.41±0.06	0.41±0.04	N.S.

approached but did not achieve significance.

Resting B-endordphin levels were very similar in runners and non-runners (1.85±Ø.46 vs. 1.83±Ø.9Ø pg/ml) as shown in table 9. Likewise, there were no significant differences in resting cortisol levels between groups at baseline (84.2±13.7 vs. 1Ø2.Ø±27.4 ng/ml; controls vs. runners) or throughout the six hour period (table 11). In addition, cortisol levels did not change significantly within a group during the six hour collection (figures 8 and 9). There was also no significant correlation between these resting cortisol and B-endorphin levels in either group (table 12).

Computer analysis of gonadotropin pulsatility was accomplished by both the PULSAR Method (Merriam, 1984a) and the program of Santen and Bardin (1973). The results of each of these methods are found in tables 13 and 14. Mean LH levels were significantly higher in runners as compared to controls (4.37±1.Ø1 vs. 1.46±Ø.2Ø mIU/ml) (figure 1Ø and table 13). The frequency of LH pulses was similar in both groups, 2.11±Ø.65 vs. 2.38±Ø.65 pulses per six hours (controls vs. runners) by the PULSAR method and 5.33±Ø.41 vs. 4.25±Ø.48 pulses per six hours (controls vs. runners) by the Santen and Bardin technique. Pulse amplitude was significantly higher in runners compared to non-runners by PULSAR (3.5Ø±Ø.44 vs. 1.45±Ø.58 mIU/ml; $p<Ø.Ø5$). Pulse amplitude was also higher in runners by the Santen and Bardin method but not significantly so (8.Ø6±1.46 vs. 2.86±Ø.44 mIU/ml; $p<Ø.Ø6$ runners vs. controls). The Santen and Bardin

Table 11 Time course of cortisol in controls and runners at rest with and without one hour of prior exercise. Cortisol levels were not different at any time point among the three studies.

Table 11

SERUM CORTISOL LEVELS AT REST

	MEAN±SEM ng/ml	Minutes					
		0	60	120	180	240	360
1.	Non-Runners	84.2±13.7	69.0±10.2	82.4±18.8	90.5±17.0	86.8±15.2	57.1±10.9
2.	Runners Rest	102.0±27.4	70.0± 0.0	84.4±22.6	83.6±11.8	92.3±13.2	88.8±13.6
3.	Runners Exercise	66.0±20.1	98.3±17.6	82.9±14.7	93.8±20.6	92.1± 8.3	67.4± 8.6

*There were no significant differences in serum cortisol among groups at any timepoint.

Table 12 There was a significant positive correlation between post treadmill levels of cortisol and B-endorphin.

Table 12

COMPARISON OF SERUM B-ENDORPHIN IMMUNOREACTIVITY AND CORTISOL

COMPARISON	CORRELATION COEFFICIENT	SIGNIFICANCE
1. Runners rest-early follicular phase	0.235	N.S.
2. Runners pre-treadmill	-0.198	N.S.
3. Runners post-treadmill	0.122	N.S.
4. Runners pre-1 hr run	0.150	N.S.
5. Runners post-1 hr run	0.020	N.S.
6. Controls rest early follicular phase	0.380	N.S.
7. Controls pre-treadmill	-0.108	N.S.
8. Controls post-treadmill	0.670	$p<0.05$

Figure 8 The time course of serum cortisol over a six hour period in runners and controls at rest. There were no differences in cortisol levels between groups.

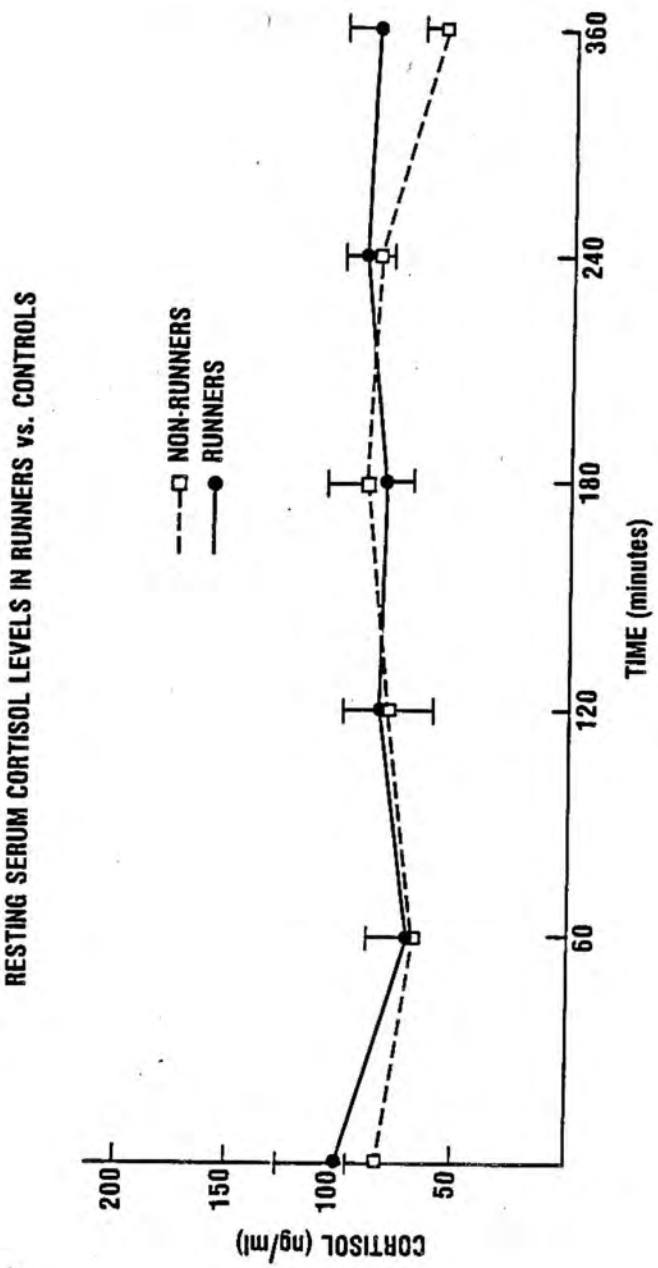

RESTING SERUM CORTISOL LEVELS IN RUNNERS vs. CONTROLS
NON-RUNNERS
RUNNERS
CORTISOL (ng/ml)
200
150
100
50
60
120
180
240
360
TIME (minutes)

Figure 9 The time course of serum cortisol over a six hour period in runners at rest and following a one hour submaximal run. There were no changes in serum cortisol due to exercise.

SERUM CORTISOL LEVELS IN RUNNERS AT REST AND AFTER A ONE HOUR TRAINING RUN

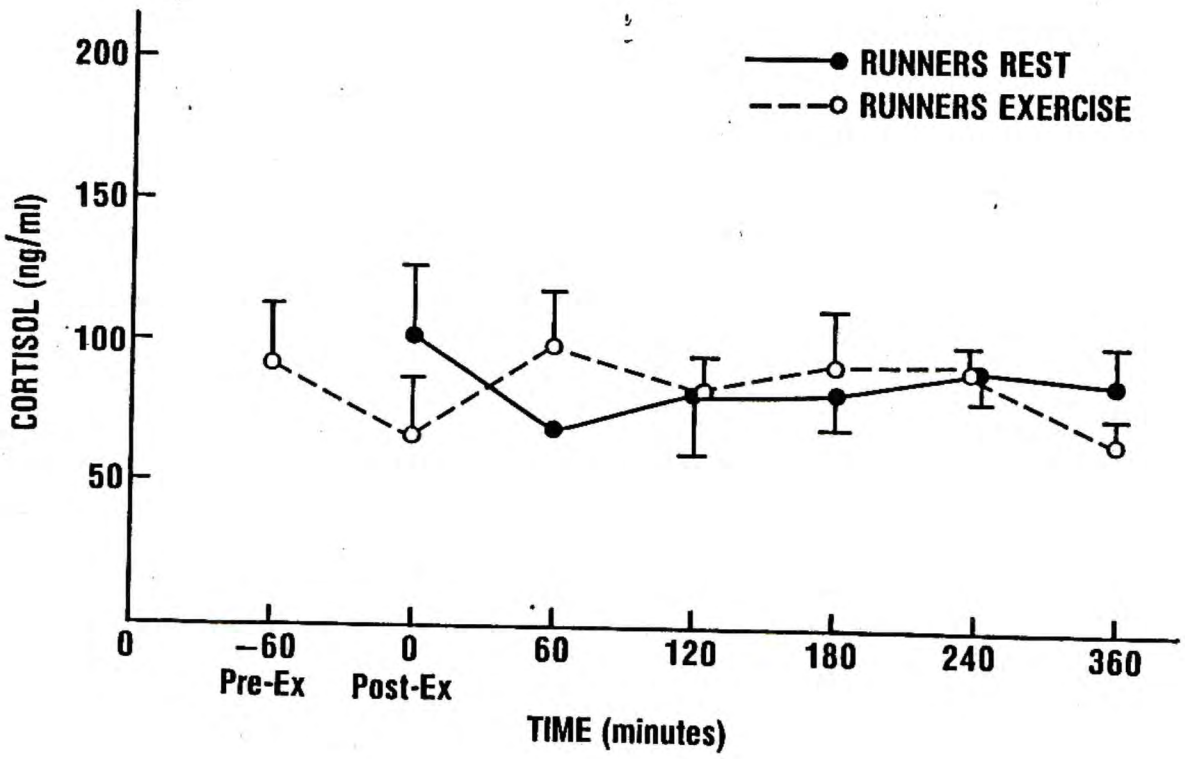


Table 13 A comparison of the PULSAR and Santen and Bardin techniques for LH pulse analysis in this study.

Table 13

MEAN SERUM LUTEINIZING HORMONE LEVELS, PULSE FREQUENCY, AND PULSE AMPLITUDE BY THE PULSAR AND SANTEN AND BARDIN (S+B) METHODS

	SUBJECTS MEAN±SEM (mIU/ml)	MEAN hLH	PULSAR # PULSES/ 6 HRS	PULSAR PULSE AMPLITUDE mIU/ml	S+B # PULSES/ 6 HRS	S+B PULSE mIU/ml AMPLITUDE
1.	Non-Runners	1.46±0.20[a]	2.11±0.65[a]	1.45±0.58[a]	5.33±0.41[a]	2.86+0.44[a]
2.	Runners Rest	4.37±1.01[b]	2.38±0.65[a]	3.50±0.44[b]	4.25±0.48[a]	8.06±1.46[a]
3.	Runners Exercise	3.89±0.67[b]	2.25±0.80[a]	3.24±0.72[b]	5.00±0.46[a]	7.06±1.48[a]

*a,b Figures within columns bearing different superscripts are significantly different ($p<0.05$)

Table 14 A comparison of the PULSAR and Santen and Bardin techniques for FSH pulse analysis in this study.

Table 14

MEAN SERUM FOLLICLE STIMULATING HORMONE LEVELS, PULSE FREQUENCY, AND PULSE AMPLITUDE BY THE PULSAR AND SANTEN AND BARDIN (S+B) METHODS

	MEAN±SEM (mIU/ml)	MEAN hFSH	PULSAR # PULSES/ 6 HRS	PULSAR PULSE AMPLITUDE mIU/ml	S+B # PULSES/ 6 HRS	S+B PULSE mIU/ml AMPLITUDE
1.	Non-Runners	7.24±0.47[a]	3.00±0.62[a]	5.06±1.32[a]	5.14±0.34[a]	12.39±1.85[a]
2.	Runners Rest	7.08±1.18[a]	3.14±0.59[a]	4.10±0.78[a]	4.57±0.81[a]	14.22±2.58[a]
3.	Runners Exercise	7.39±0.38[a]	2.57±0.65[a]	4.09±0.80[a]	6.00±1.16[a]	17.09±2.02[a]

*a,b Figures within columns bearing different superscripts are significantly different ($p<0.05$).

Table 15 Description of the one hour treadmill run.

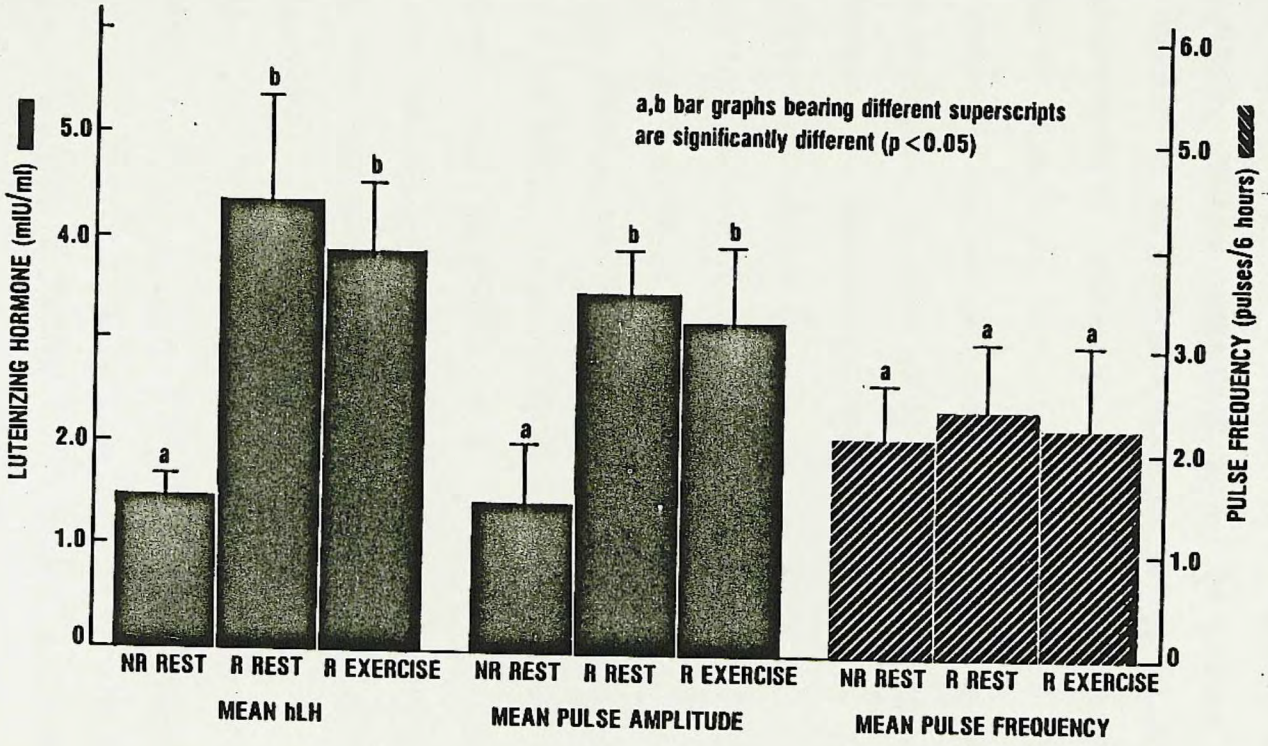
Table 15

RUNNING PERFORMANCE DURING THE ONE HOUR RUN

	MEAN ±SEM	Speed (MPH)	VO2 at 25 min (ml/kg/min)	% of VO2 max at 25 min	75% of VO2 max (ml/kg/min)	Heart Rate at 25 min (beats/min)	% of Max heart rate at 25 min	75% of Max heart rate (beats/min)
1.	Runners n=9	7.30±0.26	33.84±1.02	77.16±1.78	33.76±1.00	152.00±5.00	85.37±1.93	148.24±2.50

Figure 1Ø Resting mean serum LH levels and LH pulse amplitude were significantly higher in runners than controls. There was no difference in LH pulse frequency between groups. Neither mean LH level, pulse amplitude,or pulse frequency changed in runners as a result of a one hour training run.

SERUM LUTEINIZING HORMONE MEAN LEVEL, PULSE AMPLITUDE, AND PULSE FREQUENCY IN RUNNERS AND CONTROLS AT REST AND IN RUNNERS AFTER A ONE HOUR RUN BY THE PULSAR METHOD



technique detected 18 of 19 pulses measured by PULSAR in runners which was 56% of the total number of LH pulses detected by the Santen and Bardin method. In non-runners all of the 19 pulses measured by PULSAR were also detected by the Santen and Bardin method. These comprised 19 of 28 pulses or 48% of the total number of LH pulses detected using Santen and Bardin. Representative examples of LH pulsatility studies are provided in figure 11.

Mean follicle stimulating hormone levels were nearly the same in runners and controls (7.Ø8±1.18 vs. 7.24±Ø.47 mIU/ml) (figure 12). Likewise, by the PULSAR method there were no differences in either FSH pulse frequency (3.ØØ±Ø.62 vs. 3.14±Ø.59 pulses per six hours) or amplitude (5.Ø6±1.32 vs. 4.1Ø±Ø.78, controls vs. runners). These relationships also held true for the parameters of FSH pulsatility measured by the Santen and Bardin method; pulse frequency was (5.14±Ø.34 vs. 4.57±Ø.81 mIU/ml) and amplitude was (12.39±1.85 vs. 14.22±2.58 mIU/ml) in controls versus runners, respectively. Figures 13 and 14 give examples of FSH pulsatility studies in a runner and a non-runner.

As was the case for LH, the Santen and Bardin method consistently detected more FSH pulses than did the PULSAR technique. In runners, 19 of 22 pulses detected by PULSAR were also measured by Santen and Bardin. A total of 32 FSH pulses were measured by the Santen and Bardin method in runners. This technique measured a total of 36 FSH pulses in non-runners, 58% or 2Ø of a total of 21 of which were also measured by PULSAR.

Figure 11 These examples show that the LH values in runners were much more variable around the mean compared to those of non-runners.

REPRESENTATIVE LH PULSATILITY STUDIES IN A RUNNER AND A NON-RUNNER
hLH RESTING PULSATILITY

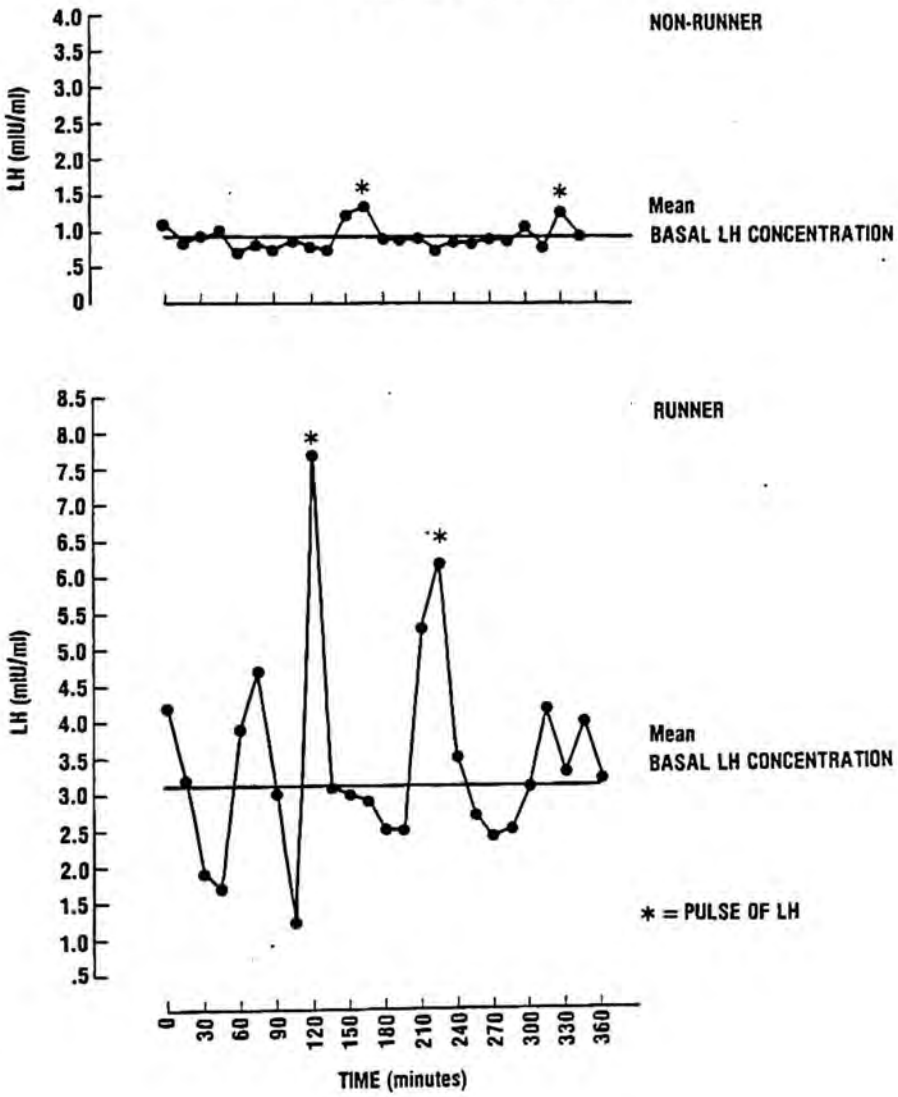


Figure 12 There were no differences in mean FSH levels, pulse amplitude, and pulse frequency between runners and controls at rest. None of these parameters changed in runners following a one hour training run.

SERUM FOLLICLE STIMULATING HORMONE MEAN LEVEL, PULSE AMPLITUDE, AND PULSE FREQUENCY IN RUNNERS AND CONTROLS AT REST AND IN RUNNERS AFTER A ONE HOUR RUN BY THE PULSAR METHOD

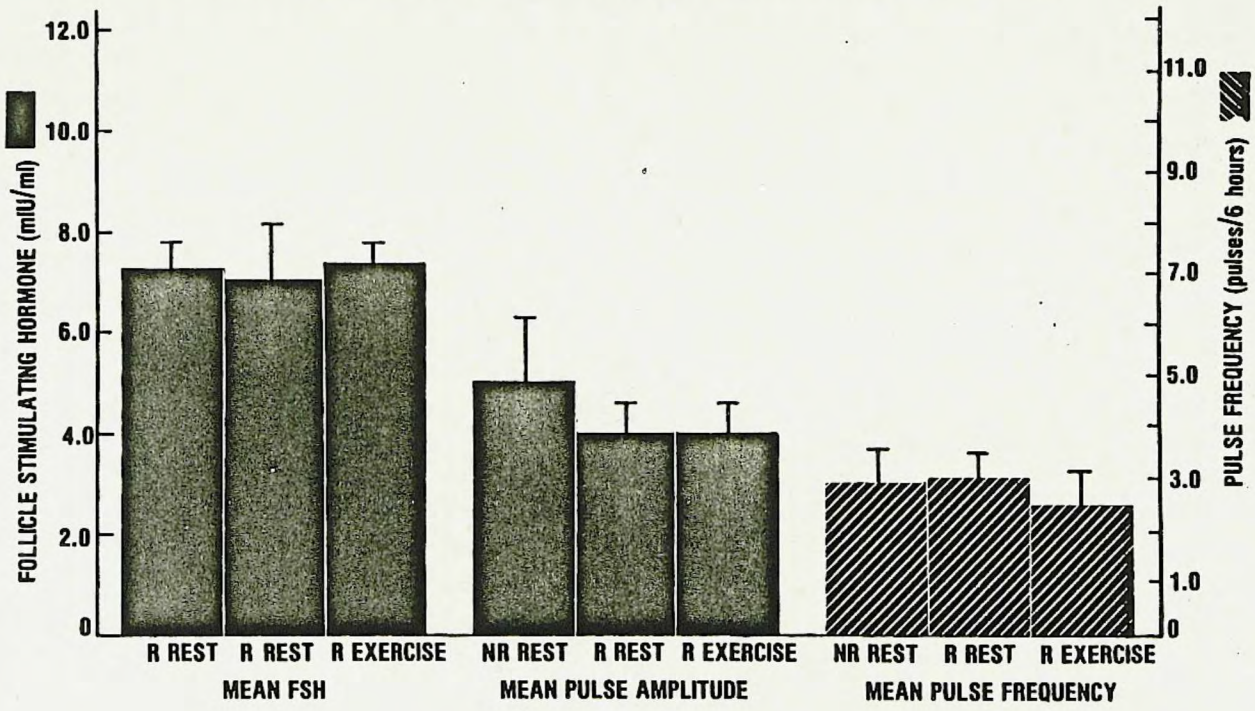


Figure 13 The FSH pulsatility studies in controls and runners were very similar.

Representative hFSH Pulsatility Study on a Non-Runner

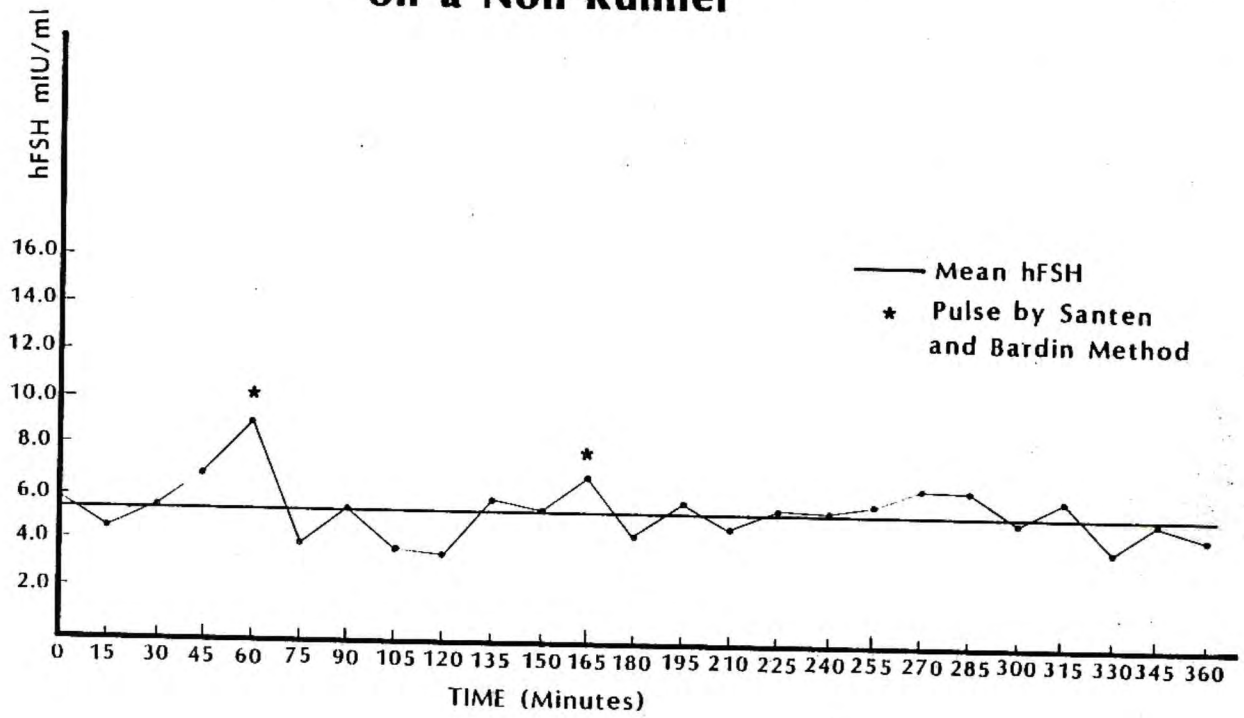
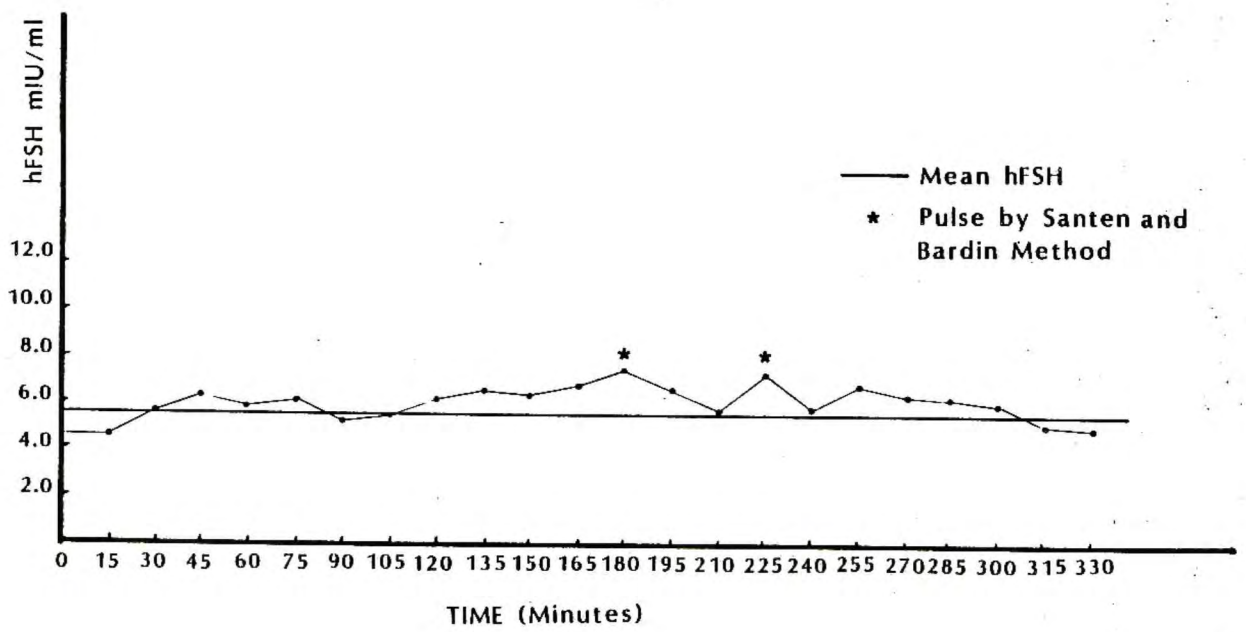


Figure 14 In runners, FSH values were much less variable around the mean as compared to LH values.

Representative hFSH Pulsatility Study on a Runner



Luteinizing hormone pulses were not coincident with follicle stimulating hormone pulses by either technique. In most subjects one or two LH pulses occured at the same time as an FSH pulse but no clear relationship existed. This lack of synchrony between LH and FSH pulses was no different in runners than controls.

D. THE EFFECT OF A TRAINING RUN ON GONADOTROPIN PULSATILITY

Runners reported back to the laboratory approximately one month following their resting studies and during the early follicular phase of their cycles. A pre-exercise baseline blood sample was drawn and then each woman performed a one hour training run on the treadmill. Immediately following the run subjects were seated, an indwelling intravenous catheter was inserted, and a blood sample was drawn. Afterwards, blood was taken every 15 minutes for six hours. This portion of the study was done to determine the effect of acute exercise on gonadotropin pulsatility. In addition, this study also provided the opportunity to compare the effects of submaximal exercise to maximal exercise, as described in part I, as to their effect on reproductive hormone levels, cortisol, and serum B-endorphin.

Subjects were to perform the one hour run at a workload equal to 75% of maximum oxygen consumption. As can be seen in table 15, runners actually exercised at 77.16$\pm$1.78% of

maximum oxygen comsumption which was equivalent to 33.84±1.Ø2 ml/kg/min. The speed of each woman's run was determined by her normal training speed, as reported on her running history, and by her heart rate at 75% of maximum oxygen consumption during the maximum treadmill test. The speed of the treadmill was gradually increased over the first 1Ø to 12 minutes to allow subjects to warm up. A typical progression was as follows:

TIME	SPEED	HEART RATE (BEATS/MIN)
1 min	5 mph	117
2-7 min	6 mph	116
7-1Ø min	6.5 mph	129
1Ø-12 min	7 mph	14Ø
12-6Ø min	7.5 mph	142=Target Heart Rate

Subjects exercised at an average speed of 7.3Ø±Ø.26 miles per hour during the one hour run. Mid-way through the run their heart rates averaged 152.ØØ±5.ØØ beats per minute which was 85.37±1.93% of maximum heart rate. Respiratory exchange ratios remained below 1.ØØ in most cases which indicated that subjects were exercising aerobically. In several cases both heart rate and respiratory exchange ratio began to increase gradually during the latter third of the run. In one case, a subject only ran for 5Ø minutes due to possible chest pain not accompanied by EKG changes, which was later diagnosed as a muscle spasm.

Hematocrit did not change significantly after the one

hour run (41.4±1.8 vs. 39.9±2.Ø pre vs. post, table 5). There was a significant increase in estradiol (88.Ø vs. 114.2, S.E. 5.1 pg/ml, pre vs. post) after the one hour run (figure 15). Progesterone and testosterone did not change significantly as a result of the run (P: Ø.82 vs. Ø.9Ø, S.E. Ø.Ø8 ng/ml; T: Ø.56 vs. Ø.74, S.E. Ø.18, ng/ml, pre vs. post table 16). Correction for post-exercise changes in hematocrit did not alter the significance of these results.

Neither B-endorphin (1.21±Ø.42 vs. 1.32±Ø.7Ø pg/ml pre vs. post) nor cortisol (91.8±22.1 vs. 66.Ø±2Ø.1 ng/ml pre vs. post) changed significantly as a result of the one hour run (tables 8 and 9; figures 16). In fact, when post-exercise values were corrected for changes in hematocrit, both B-endorphin (Ø.66±Ø.21 pg/ml) and cortisol (69.4±26.4) values were actually lower as compared to pre-exercise levels. However, there were no significant correlations between B-endorphin and cortisol either before (r=Ø.15Ø) or after (r=Ø.Ø2) the one hour run (table 12).

The parameters of gonadotropin pulsatility were quite similar in runners at rest as compared to runners who were resting following a training run. Mean LH levels were 3.89±Ø.67 mIU/ml which was significantly different than mean LH values in non-runners, but only slightly lower than mean LH values in runners without exercise (table 13). According to the PULSAR method, 2.25±Ø.8Ø pulses per 6 hours was similar to values for controls and runners at rest, whereas LH pulse amplitude (3.24±Ø.72 mIU/ml) was significantly

Table 16 Estradiol and testosterone levels rose significantly in runners following a training run. Paired t-tests were used to calculate significance.

Table 16

CHANGES IN STEROID CONCENTRATIONS IN RUNNERS AFTER A ONE HOUR RUN

	Pre Exercise	Post Exercise	Difference (Pre-Post)	Standard Error	Post Exercise Corrected for Hct.
1. Estradiol (pg/ml)	88.0	114.20	-26.20	5.10	139.30
2. Progesterone (ng/ml)	0.82	0.90	-0.08	0.08	1.02
3. Testosterone (ng/ml)	0.56	0.74	-0.18	0.11	0.73

Figure 15 Serum estradiol increased significantly in runners following a one hour training run.

MEAN STEROID HORMONE CONCENTRATIONS OF RUNNERS BEFORE AND AFTER A ONE HOUR RUN AT 75% MAXIMUM OXYGEN CONSUMPTION

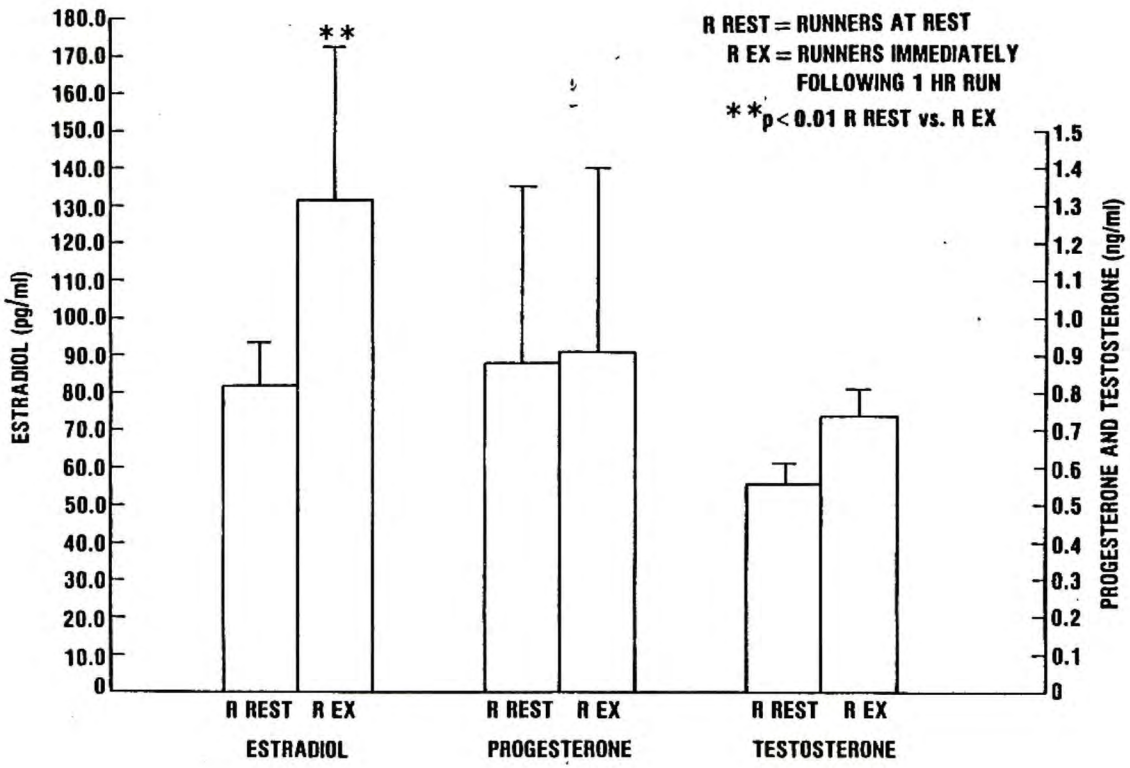
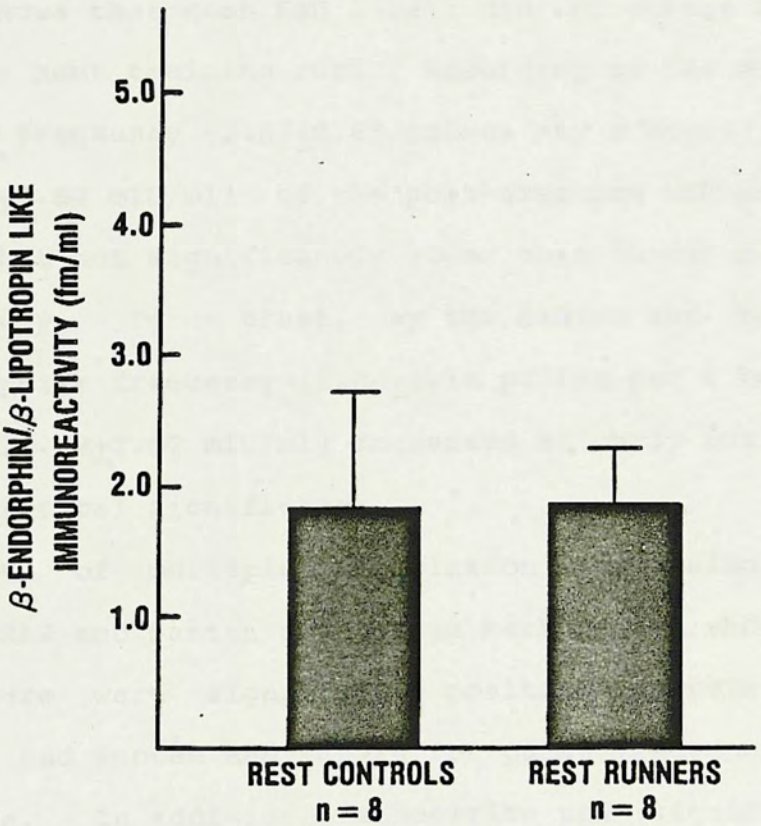


Figure 16 There were no differences in resting levels of serum B-endorphin1-31 between groups.

EARLY FOLLICULAR PHASE SERUM β-ENDORPHIN IMMUNOREACTIVITY AT REST IN RUNNERS AND CONTROLS



higher than that of non-runners but no different from that of runners at rest.

Neither LH pulse amplitude (7.Ø6±1.48 mIU/ml) nor LH pulse frequency (5.ØØ±Ø.46 pulses per 6 hours) were different when results from exercise pulsatility studies were compared to resting studies of runners or controls measured by the Santen and Bardin method. This method determined 13 of a total of 18 LH pulses measured by PULSAR which was 38% of all pulses detected by the Santen and Bardin technique.

Table 14 shows that mean FSH levels did not change as a result of a one hour training run. According to the PULSAR method both the frequency (2.57±Ø.65 pulses per 6 hours) and amplitude (4.Ø9±Ø.8Ø mIU/ml) of the post-exercise FSH pulses were slightly but not significantly lower than those during the resting study. In contrast, by the Santen and Bardin program, FSH pulse frequency (6.ØØ±1.16 pulses per 6 hours) and amplitude (17.Ø9±2.Ø2 mIU/ml) increased slightly but did not attain statistical significance.

The results of multiple correlation regressions to compare the PULSAR and Santen and Bardin Methods are shown in table 17. There were significant positive correlations between PULSAR and Santen and Bardin for pulse frequency and pulse amplitude. In addition, a positive and significant correlation existed between LH pulse frequency and amplitude ($r=Ø.642$, $p<Ø.Ø5$) and FSH pulse frequency and amplitude ($r=Ø.719$, $p<Ø.Ø5$) for the PULSAR but not the Santen and Bardin method.

Table 17 There was a good correlation between the PULSAR and Santen and Bardin methods of pulse analysis.

Table 17

COMPARISONS OF THE PULSAR (P) AND SANTEN AND BARDIN (S+B) METHODS TO ANALYSE GONADOTROPIN DATA IN THIS STUDY

	Comparison	Correlation Coefficent	Significance
1.	#LH Pulses by S+B	0.670	$p<0.05$
2.	LH Pulse Amplitude by P vs. S+B	0.480	$p<0.05$
3.	LH Pulse Amplitude vs. #LH pulses by P	0.642	$p<0.05$
4.	LH Pulse Amplitude vs. #LH Pulses by S+B	0.371	N.S.
5.	#FSH Pulses by P vs. S+B	0.821	$p<0.05$
6.	FSH Pulse Amplitude vs. #FSH Pulses by P	0.719	$p<0.05$
7.	FSH Pulse Amplitude vs. #FSH Pulses by S+B	0.011	N.S.

V. DISCUSSION

Overall, the results of this study indicate that long-term endurance running in female athletes leads to abnormal gonadotropin release which is manifested by high mean LH levels and high LH pulse amplitudes both at rest and after exercise. Exercise-associated changes in gonadotropin release do not appear to be associated with chronically elevated resting B-endorphin levels, at least as reflected by peripheral serum levels, or by an exaggerated B-endorphin response to maximal exercise. These data demonstrate that a typical training run evokes increases in both serum estradiol and testosterone in trained runners. Moreover, endurance trained athletes have decreased early follicular phase serum estradiol levels at rest and also subnormal luteal phase progesterone levels. Results from this study suggest that the acute increases in reproductive steroid levels accompanying daily training runs, combined with chronically depressed mean serum estradiol levels, may result in a disruption of the normal steroid negative feedback control of gonadotropin release.

A. COMPARISON OF THE CHARACTERISTICS OF THIS STUDY POPULATION WITH THOSE IN SIMILAR STUDIES

No one is certain how many miles of running per week and at what intensity are necessary to evoke changes in the menstrual cycle. Furthermore, it is debatable whether there is a certain threshold combination of training intensity and duration above which all women develop menstrual irregularities, or whether the threshold level is different in each individual. It is also possible that there exists a certain subset of female athletes who are more highly susceptible to the development of exercise-associated menstrual disorders than other athletes.

The runners in this study were training an average of more than 50 miles per week which is equal to or greater than the number of miles run by amenorrheic or eumenorrheic women in most other studies. The fact that their average maximum oxygen consumption of 45.0±1.3 ml oxygen/kg/min was in the excellent range for females on the Bruce Protocal (American Heart Association, 1972) was evidence that this population was highly trained. In addition, runners had training bradycardia and lower maximum heart rates compared to non-runners, both of which are effects of the increased vagal tone that accompanies endurance training (McArdle, 1981). None of the control subjects had exercised regularly for the past year, although one subject had previously been a long distance runner. Most of the sedentary women volunteered in order to get baseline fitness measurements before beginning

exercise programs. The mean maximum oxygen consumption of 28.7±1.4 ml oxygen/kg/min for this group was in the "fair" range for the Bruce Protocol and within the normal range of 25-35 ml/kg/min for sedentary women (McArdle, 1981). The fact that the controls had higher respiratory exchange ratios than trained runners post-treadmill has also been reported by Issekutz, (1961) and may indicate that trained athletes have a more efficient system for buffering exercise-stimulated lactic acid accumulation.

Many have suggested that runners have a later age at menarche than non-runners (Frisch, 1981; Calabrese, 1982). In this study, the runners' age at menarche (12.93±Ø.25 years) was slightly later but not statistically different from that of controls (11.28±1.28 years). In addition both groups had normal menstrual cycle lengths.

The runners in this study had few of the risk factors associated with the development of amenorrhea. The incidence of menstrual irregularities seems to be highest in the young, nulliparous athlete, especially in those who begin to exercise prior to menarche. Five of the nine runners had a proven history of fertility as evidenced by prior pregnancies. None of the runners had begun running before menarche although one was an avid tennis player in her childhood. Several authors have found that athletes over 3Ø years of age are less likely to develop exercise associated menstrual irregularities (Speroff, 198Ø). The runners in this study averaged 32.5±1.6 years of age.

Although seemingly lean subjects were selected as controls, there was still a difference in percent body fat between the two groups. The non-runners in this study had 24.4±2.3% body fat by hydrostatic weighing which is within the normal range for the typical American adult female (Frisch, 1976). However, the runners had 16.4±7.5% fat by underwater weighing or 13.5±1.1% by the skinfold technique. Both of these values are below the 17% body fat which Frisch (1974) believes is necessary for the onset of menses, and well below the 22% body fat which, in her opinion, is necessary for menstrual cycle maintenance. Some authors speculate that since adipose tissue is the site for the peripheral conversion of androgens to estrogens, a decreased body fat may result in an imbalance in these steroid hormones and in their feedback to the pituitary or hypothalamus (Loucks, 1984). More recent studies suggest that decreased body fat alone is not responsible for the development of menstrual disorders. The runners in this study had body fat levels within the range found for amenorrheic runners, 14.1±1.2% (Baker, 1981) to 20.4±1.1% (Dixon, 1984) and also for eumenorrheic runners, 15.9±6.6% (Yu-Yahiro, 1986) to 22.1±3.3% (Rokainen, 1985b).

B. INVESTIGATION OF BASAL HORMONE LEVELS IN TRAINED RUNNERS AND SEDENTARY CONTROLS

Amenorrheic runners are anovulatory and display no

menstrual bleeding. They have steroid hormone profiles which resemble early follicular levels but do not change throughout the month; some amenorrheics even have serum estradiol values below normal early follicular phase values (20-60 pg/ml) (Wilson, 1985). In addition, amenorrheic runners have a reduced frequency of GnRH secretion (Veldhuis, 1985a) manifested by a decreased LH pulse frequency. If eumenorrheic runners are found to have some subtle disruption of normal cyclicity or suboptimal hormone levels which are not severe enough to cause amenorrhea, an understanding of the process by which menstrual irregularities develop in athletes might be gained.

The eumenorrheic runners in this study had seemingly normal menstrual cycles displaying monthly bleeding and having cycles of normal length. However, basal blood samples revealed that serum estradiol levels were significantly lower in runners as compared to controls. As in our study, Cumming (1985b) and Rokainen (1985b) reported lower estradiol levels in eumenorrheic runners as compared to controls although these differences were not significant. Fears (1982) also studied eumenorrheic runners during the early follicular phase of their cycles and measured mean serum estradiol levels of 61.5 ± 6.5 pg/ml which are comparable to the mean estradiol values of 68.0 ± 4.0 measured in this study.

Whether exercise has a direct effect on ovarian steroidogenisis cannot be determined from these data. However, in typical ovarian failure baseline LH and FSH

values are elevated reflecting a lack of feedback inhibition by estrogen (Greenspan, 1983). Runners in this study had significantly elevated mean LH levels compared to controls.

Boyden, (1984) demonstrated a progressive decrease in plasma estradiol as women increased their weekly mileage in preparation for a marathon. He also observed a decrease in gonadotropin response to exogenous GnRH. However, there were no significant correlations between estradiol levels and decreased gonadotropin response. Therefore, Boyden hypothesizes that decreased hypothalamic/pituitary responsiveness is the initial effect of running which then causes lower serum estradiol levels.

Previous studies have found serum testosterone values to be higher in runners than controls (Dale, 1979a; Ronkainen, 1985a). It was suggested that in these lean athletes insufficient body fat was available for the peripheral aromatization of androgens to estrogens. Despite the fact that runners had significantly lower body fats than non-runners, serum testosterone levels in this study were virtually identical.

Follicular phase progesterone levels were no different between groups and were all within the normal range for that phase (Ø.1-Ø.9 ng/ml; Hazelton Laboratories). However, the four runners who were in their luteal phases during the treadmill test had a mean progesterone value of 1.2±Ø.3 ng/ml which is below the normal luteal phase range of 1.5 to 2.Ø ng/ml (Hazelton Laboratories). Reduced luteal phase progesterones have also been reported in runners (Shangold,

1979) and swimmers (Bonen, 1981).

It is the granulosa cells of the corpus luteum which produce progesterone during the luteal phase. Lack of sufficient estradiol stimulation during the follicular phase could result in abnormal development of the ovarian follicles and of the endometrium. This might include a decrease in the number of granulosa cells and in the size of the theca cells. In addition, if estradiol levels are low, as was found in the runners, there may be fewer LH receptors on the granulosa cells. Luteinization of the granulosa cells after ovulation is stimulated by the binding of LH to these receptors. Then LH stimulates estradiol and progesterone release from luteinized theca and granulosa cells which is necessary for proliferation of the endometrium.

Unfortunately, ovulation was not documented in this study so there is no way to determine the exact luteal phase length in these women. Prior (1982) reported a high incidence of shortened luteal phase lengths among women training for a marathon. It is possible that runners in this study had severly shortened luteal phases or anovulatory cycles such that progesterones measured actually reflected late follicular values. However, this is highly unlikely since three of four women were between days 22 and 28 of their cycles and since their luteal phase estradiols averaged 264.5 ± 79.5 pg/ml, a typical value for that phase of the cycle. At this time no data are available to confirm or refute any of these theories.

C. STEROID HORMONE RESPONSES TO MAXIMAL AND SUBMAXIMAL EXERCISE

Exercise-induced changes in reproductive steroids may reflect changes in the rates of production, metabolism or clearance during exercise. There appears to be a differential response to exercise in the follicular compared to the luteal phases (Shangold, 1981; Jurkowski, 1978). Also, these responses may be intensity dependent (Bonen, 1984) and may be different in trained compared to untrained athletes (Keizer, 1980).

During exercise there is a significant shunting of blood flow to the working muscles and away from the gut and mesenteric region (Asstrand, 1977). Studies have demonstrated decreased hepatic (Rowell, 1964) as well as renal (Radigan, 1949) blood flow during exercise. This is important since the steroids are metabolized in the liver and excreted in the urine. In addition, Keizer (1980) demonstrated decreased metabolic clearance of estradiol after exercise. He administered radio-labelled estradiol by the constant infusion technique into six untrained women prior to ten minutes of bicycle exercise at 75% of maximum oxygen consumption. Results showed a mean decreased metabolic clearance rate of 36% which ranged from 18 to 67%.

Estradiol increased significantly in runners in the present study following the one hour run at 75% of maximum oxygen consumption. However there was no increase in estradiol in either runners or controls as a result of maximal exercise. There were also no significant changes in estradiol after treadmill exercise when women were grouped according to phase of their menstrual cycles. In contrast, studies have found increases in serum estradiol after short term intense or maximal exercise in untrained women (Jurkowski, 1978; Keizer, 1980) and trained women (Spitler, 1983). Prolonged submaximal exercise by trained runners in the early follicular phases has been found to both increase (Pepper, 1983) or not change (Spitler, 1983; Loucks, 1984) serum estradiol levels.

However, there are several lines of evidence that increased steroid production during exercise cannot be attributed to decreased clearance and metabolism alone. The first is that there are different exercise-induced increases in steroid hormones during the follicular and luteal phases. For instance, testosterone increased 54% during the luteal phase and only 21% during the follicular phase in recreational runners after a 30 minute run (Shangold, 1981). Also, the progesterone response to exercise is much greater in the luteal than follicular phase (Jurkowski, 1978; Bonen, 1983). It is unlikely that the metabolic clearance rate changes with different phases of the cycle. Bonen (1983) has shown no change in nutrient metabolism in the follicular

versus the luteal phase.

It is also feasible that exercise could stimulate increased adrenal cortex and/or ovarian production of steroids. Exercise as well as other forms of stress have been demonstrated to increase adrenal cortisol production (Loucks, 1984). However, in one study serum androgen increased post-exercise despite adrenal supression by dexamethasone (Sutton, 1974).

This study found no changes in serum progesterone either at maximal or submaximal exercise. These findings are in part explained by the fact that two previous studies have found increases in progesterone in the luteal but not the follicular phases (Jurkowski, 1978; Bonen, 1983). However, when women who performed the treadmill test during the luteal phase were analysed alone, there was still no apparent exercise-induced increase in progesterone.

Serum testosterone has been shown to increase in both trained (Shangold, 1981) and untrained (Wallace, 1983) women following exercise and in response to both prolonged aerobic (Shangold, 1981) and anaerobic (Wall, 1983) exercise. This study found no change in testosterone in either runners or controls following maximal exercise. However, serum testosterone levels increased 32% following a one hour submaximal run. These results are comparable to the 54% increase in testosterone seen post-exercise in joggers during the follicular phase (Shangold, 1981). They are in contrast to the results of Loucks (1984) who found no increase in testosterone after an intense 40 minute run in trained

runners.

Whether the increased estradiol and progesterone values following prolonged exercise result from increased production or decreased metabolism and clearance, cannot be determined from these data. Regardless, it is the increased serum level of these steroids that are being detected by the pituitary and the hypothalamus. Perhaps the daily "spikes" in estradiol and testosterone following training sessions, repeated frequently, may set the stage for, or participate in the endocrine changes which result in amenorrhea.

D. EFFECTS OF MAXIMAL AND SUBMAXIMAL EXERCISE ON SERUM BETA-ENDORPHIN LEVELS

As in other studies, B-endorphin levels rose significantly in response to maximal treadmill exercise in both trained runners and controls. Berk (1981) also studied trained and untrained females and found that B-endorphin rose after a maximum treadmill test in both groups with no difference in the magnitude of the rise between groups. The percent rise in B-endorphin over baseline tended to be greater in the control group although the amount of absolute work done by runners was greater. These results support the findings of Colt (1981) that the percent rise in B-endorphin over baseline is negatively correlated with the number of years of training.

The finding that B-endorphin levels did not rise in trained runners after a one hour run at 75% of maximum oxygen consumption was at first surprising. However, a review of other studies seems to show that in trained athletes, exercise below anaerobic threshold causes little or no increase in serum B-endorphin levels (Colt, 1981). The runners in this study were exercising below anaerobic threshold as evidenced by their respiratory exchange ratios which were below 1.ØØ. When five trained males and one female runner performed a 3Ø minute run at 6Ø% of maximum oxygen consumption, there was no significant rise in serum B-endorphin (Farrell, 1982). Similarly, there was only a 49% increase in B-endorphin after an easy training run (Colt, 1981) in trained runners, 2Ø males and six females. Generally, the rise in serum B-endorphin post-exercise is greater in males than females. In Colt's study, men and women were grouped together. This fact may explain why a rise in B-endorphin was observed in his study after submaximal exercise, but not in ours. Perhaps the stress of oxygen debt which accompanies anaerobic exercise is part or all of the stimulus for the increase in B-endorphin release with strenuous exercise, at least in athletes.

In addition, differences in core temperature during exercise might explain the differential responses of B-endorphin to maximal and submaximal exercise. Viswanathan (1985) found that B-endorphin levels rose more after exercise at 22 degrees centigrade than at 5 degrees centigrade in

eumenorrheic women and men. Although in the present experiment ambient temperature was constant for both maximal and submaximal exercise, it is likely that core temperature was highest following maximal exercise. In addition, trained athletes have better thermoregulatory mechanisms than sedentary controls (Astrand, 1977). As a training adaptation they have a lower threshold at which sweating begins and therefore endurance athletes dissipate heat more efficiently. It is conceivable that non-runners had higher core temperatures at maximal exercise than runners which stimulated greater increases in B-endorphin levels post-exercise. However, this point is speculative since core temperatures were not measured in this study.

Finally, B-endorphin is considered a stress hormone because it rises in response to various forms of stress such as electric shock (Hulse, 1983) and heat (Viswanthan, 1985) and rises simultaneously with other stress hormones like cortisol (Carr, 1981) or norepinephrine (Bortz, 1981). It is therefore conceivable that the maximal treadmill test was perceived as stressful, a "test" of the success of hours of training and a measure of one athlete's performance in comparison to another, while the one hour run was similar to training runs which the athletes did daily. This difference in perceived stress might also explain the lack of rise in B-endorphin after submaximal exercise in runners.

E. EFFECTS OF EXERCISE ON SERUM CORTISOL LEVELS

Most studies have found an increase in cortisol levels following maximal bicycling or treadmill exercise (Farrell, 1983; Maehulum, 1986; Cumming, 1983). However, this study failed to find a significant rise in cortisol after maximal exercise in either group although cortisol levels did rise slightly in all controls and in all but two runners. These results cannot be explained by assay variability since intra-assay coefficient of variation was only 2.9%. However, for most subjects the maximum treadmill test constituted their first visit to the laboratory. Perhaps the preparation for the treadmill test including the physical exam, the venous puncture, or the description of the test itself caused sufficient stress to raise pre-exercise cortisol levels. Although pre-exercise values were within the normal range of cortisol values sampled in the morning, 40-240 ng/ml (Wilson, 1985), they were higher than mean cortisol values for both controls and runners during the resting pulsatility study (controls 106.0±18.8 vs. 80.04±13.7; runners 146.0±26.3 vs. 89.98±27.40 pre-exercise vs. resting pulsatility study).

Cortisol samples measured hourly over the six hour pulsatility studies showed no diurnal variations from morning to afternoon, and no differences were found between runners and controls at rest or in runners at rest with and without a preceeding bout of exercise. If resting cortisol levels were chronically elevated in runners, or if daily exercise caused significant increases in serum cortisol, this might be

expected to effect gonadotropin or steroid levels as has been demonstrated in previous studies (Doerr, 1976; Cumming, 1983). However, in this study changes in gonadotropin pulsatility and ovarian steroids in runners cannot be attributed to altered cortisol levels.

As with B-endorphin levels, serum cortisol decreased slightly in runners following a one hour training run. Few (1974) found that cortisol levels decreased after "light" exercise in trained runners. Although a one hour run at 75% of maximum oxygen consumption would not be considered "light" by most standards, this run was below anaerobic threshold and perhaps did not reach the critical level necessary to trigger a massive release of cortisol from the adrenal cortex.

It might be expected that cortisol and B-endorphin levels would change in a parallel fashion since both B-endorphin and ACTH are derived from the same precursor molecule, proopiomelanocortin. However, there were no significant correlations between B-endorphin and cortisol either at rest or in response to maximal or submaximal exercise. These findings are in agreement with Carr (1981). He showed that both ACTH and B-endorphin/B-lipotropin immunoreactivity rose in a parallel fashion after exercise in males but cortisol levels did not change. In addition, Appenzeller (1983) states that plasma cortisol takes approximately 15 minutes to rise after the initiation of exercise. Since the mean times on the treadmill for runners (14.7±Ø.6 min) and controls (1Ø.3±1.2 mins) were both less than fifteen minutes, it is conceivable that a maximum

treadmill test by the Bruce Protocol is not of sufficient length to show post-exercise increases in cortisol. Finally, it is possible that during the maximum treadmill test serum B-endorphin itself attenuated the cortisol response to exercise. Beyer (1986) provides evidence for this theory by his data which show that the cortisol response to exogenous ACTH was blunted when men were pretreated with synthetic B-endorphin.

F. INVESTIGATION OF GONADOTROPIN PULSATILITY IN TRAINED RUNNERS AND CONTROLS AT REST AND IN RUNNERS WITH AND WITHOUT SUBMAXIMAL EXERCISE

The runners in this study had significantly higher mean LH levels and higher LH pulse amplitudes compared to sedentary controls. There was no difference in LH pulse frequency between the two groups and neither mean LH level, LH pulse frequency nor amplitude changed in trained runners after a one hour run.

It is quite evident that analysis of a single blood sample may misrepresent the mean LH level since it could be taken anywhere between the zenith and nadir of a pulse. However, few studies report mean LH values which are determined by taking the average of a series of samples over time. One study did sample women at 20 minute intervals for

24 hours and found a mean LH level of 8.ØØ±1.4Ø mIU/ml (Veldhuis, 1986). This is higher than the mean LH values in the present study which were 4.37±1.Ø1 mIU/ml and 1.46±Ø.2Ø mIU/ml in runners compared to controls. The LH values for subjects in this study are comparable to follicular phase LH values determined by a single baseline sample which were 2.6±1.3 mIU/ml for controls and 2.3±1.3 mIU/ml eumenorrheic runners (Chang, 1986). Chang also found mean FSH values of 8.Ø±4.4 mIU and 9.Ø±1.9 mIU for controls and runners which were similar to the FSH values calculated for runners (7.Ø8±1.18 mIU/ml) and controls (7.24±Ø.47 mIU/ml) in the present study.

The luteinizing hormone pulse frequencies measured in all groups were comparable to the results of other investigators who used similar sampling protocols and methods of analysis. A generalization can be made from the studies reviewed in this paper that, utilizing the same technique for pulse analysis, the more frequent the rate of sampling, the greater will be the number of pulses detected. For example, Reame (1984) used a modified Santen and Bardin technique and found 7.7±Ø.6 pulses per 12 hours with a 2Ø minute sampling interval and 11.8±Ø.6 pulses per 12 hours with a 1Ø minute interval. The present study used a sampling interval of 15 minutes and, by the Santen and Bardin technique, attained an LH pulse frequency in runners at rest of 4.25±Ø.48 pulses in 6 hours, a value which falls between Reame's 1Ø and 2Ø minute findings. By the PULSAR technique, women in this study had slightly greater than two LH pulses in a six hour period.

This value is slightly greater than the 8.Ø±1.4 LH pulses per 24 hours found by Brody (1984). He also examined women in the early follicular phase sampling every 2Ø minutes and performed pulse analysis by PULSAR.

Many studies on gonadotropin pulsatility elected to measure only LH, not FSH (Cumming, 1985 A and B; Veldhuis, 1985a) or failed to find FSH pulses in some of their subjects (Reame, 1984). One author (Fisher, 1986) did measure FSH pulsatility in women during the early follicular phase defining a pulse as a multiple of the intraassay coefficient of variation. He found an FSH pulse frequency of 3.5±Ø.8 pulses per 4 hours and an FSH pulse amplitude of 1.5±1.2 mIU/ml. In comparison, sedentary women in this study had slightly fewer FSH pulses (3.ØØ±Ø.62 pulses per 6 hours) and higher FSH pulse amplitudes (5.ØØ±1.32 mIU/ml) by the PULSAR method.

Cumming (1985 A and B) measured LH pulse frequency and amplitude in eumenorrheic joggers and sedentary controls. He used a similar sampling regimen, drawing blood every 15 minutes for 6 hours during the early follicular phase, and performed pulse analysis by the Santen and Bardin technique. The range of values for pulse amplitude and frequency were very comparable to those in the present study. Cumming found significantly fewer LH pulses in joggers (2.5 pulses per 6 hours) compared to controls (4.5 pulses per 6 hours). By this same method of pulse analysis, runners at rest in this study had slightly higher LH pulse frequencies than non-

runners (4.25±Ø.48 vs. 2.68±Ø.44 pulses per 6 hours respectively).

Cumming (1985 A) also found a decreased LH pulse amplitude in joggers (2.5mIU/ml) compared to controls (7.5 mIU/ml). In comparison, pulse amplitudes calculated by the Santen and Bardin method for controls and runners in the present study were 5.33±Ø.41 and 8.Ø6±1.46 mIU/ml respectively. Similar to our results, Cumming (1985 B) found no change in LH pulse amplitude after exercise in his joggers. However, he found that LH pulse frequency decreased in joggers to about 1.25 pulses per 6 hours after a training run.

The discrepancies between data in the present study and the findings of Cumming (1985 A and B) may be explained by several factors. The first is a difference in subject population. The athletes in Cumming's study appear to be recreational joggers since he states that runners trained at least 32 km (about 18 miles) per week. In contrast, the women in the present study were highly trained elite runners, training an average of more than 5Ø miles per week. In addition, they had been training for an average of more than 8 years as compared to Cumming's population who had been running "at least 6 months". Secondly, in Cumming's study baseline serum estradiol levels were no different in runners than controls. It is possible that the changes which Cumming observed demonstrate an initial exercise-associated disruption in the hypothalamic-pituitary axis. Perhaps with prolonged disruption, this condition then leads to estradiol

levels which are sufficiently low to remove the normal degree of estradiol negative feedback at the pituitary and/or hypothalamus resulting in high amplitude LH pulses.

A third possible explanation for the contrasting findings may lie in the fact that different methods of pulse analysis were used. The present study analysed pulsatility by two methods, a modified Santen and Bardin technique which identified pulses by a multiple of the intraassay coefficient of variation, and by PULSAR which tends to give a more conservative estimate of pulsatility than the aforementioned method. Cumming performed pulse identification according to the methods of Reame (1984) which are very similar to the modified Santen and Bardin used in this study. Therefore, since differences in LH pulsatility were demonstrated by two different techniques in the present study, and since the methods of pulse analysis used were comparable to or more conservative than those used by Cumming, it seems unlikely that these conflicting findings of the two studies can be attributed to differences in methods of pulse analysis.

G. SUMMARY OF FINDINGS

This study is the first to find higher mean LH levels and higher LH pulse amplitudes in trained runners. The additional findings of this study along with the investigations of other authors provide a reasonable picture

of how this might occur. First, consider the high mean LH levels in runners. As previously mentioned, estradiol has an inhibitory effect on GnRH stimulated gonadotropin release during the early follicular phase of the menstrual cycle. Lower estradiol levels could remove some degree of the normal negative feedback allowing GnRH to stimulate the release of larger than normal quantities of LH.

Hoff (1983) hypothesized that there are two sets of neurons in the hypothalamus involved in the control of GnRH release. One is a negative-feedback loop, activated by estrogen deficiency, and one is a positive-feedback loop which is active during times of estradiol excess such as prior to ovulation. Furthermore, Hoff offers evidence that the pituitary releasing mechanism for LH is sensitized by prior exposure to GnRH. He showed that a prior low dose infusion of GnRH produced a 2.5 fold greater acute increase in LH in response to 1Ø micrograms of GnRH as compared to non-infused controls. According to his theory, low estradiol levels, such as found in the runners, could activate the hypothalamic neurons sensitive to estradiol deficiency resulting in GnRH stimulation. Therefore, prolonged periods of low estradiol levels, such as may be present in the runners, would result in high mean LH levels.

This study also found an increased LH pulse amplitude in runners but no effect on LH pulse frequency. Pulse frequency is determined primarily by the spontaneous activity of the GnRH pulse generator in the hypothalamus while circulating steroid hormones may act at the pituitary to modulate pulse

amplitude (Cumming, 1985B). Estradiol influences gonadotropin release at both the hypothalamic and pituitary levels during the follicular phase. Yen (1980) demonstrated that estradiol has a direct effect on the pituitary gonadotrophs. He showed that with constant GnRH infusion, pituitary LH production increased from 500 IU per 4 hr to 2000 IU per 4 hr from the early to late follicular phases and to 10,000 IU per 4 hr on the day before the LH surge. During the follicular phase estradiol as well as GnRH stimulates increased synthesis of the LH storage pool. However, estradiol also inhibits GnRH release. Yen suggests that these seemingly contradictory functions of LH actually work to balance the priming and releasing actions of LH so that a sufficiently large quantity of LH will accumulate in the storage pool for release during the midcycle LH surge.

Evidence that estradiol preferentially affects pulse amplitude over pulse frequency is provided by the study of Veldhuis (1984a). He administered estradiol to males over a 4.5 day period and demonstrated a significant decrease in their LH pulse amplitude with no change in LH pulse frequency. It is reasonable to assume that subnormal estradiol levels could produce the opposite effect, an increase in LH pulse amplitude with no change in pulse frequency.

Also, in a recent study, MacConnie (1986) found decreased LH pulse amplitude and frequency in male marathon runners. She also found post-exercise increases in gonadal

steroids and states that these prolonged, repetitive elevations of gonadal steroids accompanying training may explain altered gonadotropin secretion in these runners.

Furthermore, estradiol has important roles in normal follicular development and in the timing of ovulation. It might be speculated that low luteal phase progesterone could reflect improper follicular maturation, a specific defect in luteal cell steroidogenesis, or a problem in the hypothalamic-pituitary axis. In addition, subnormal progesterone levels may be indicative of luteal phase defects.

Estradiol must reach a critical level, approximately 300 pg/ml, in order to trigger the preovulatory LH surge. It is possible that subnormal follicular phase levels of estradiol could result in a longer time to reach this critical level. If the estradiol surge is somewhat below the critical level, it could cause a lower than normal LH rise and insufficient LH for normal luteinization of the corpus luteum. In addition, several factors must work in synchrony in order to trigger the preovulatory LH surge. The proper priming and releasing actions of GnRH as well as the synergistic effects of progesterone must all act simultaneously when the critical level of estradiol is reached. Failure of any of these factors to coincide could cause improper timing and perhaps a blunting of the LH surge. Since it is the LH surge which triggers the release of the ovum from the ovarian follicle i.e. ovulation, if this condition progressed to a point

where the LH surge was insufficient to cause ovulation, it could result in anovulatory cycles such as are seen in exercise-induced amenorrhea.

One of the limitations of doing a study on human subjects is that the data collected are by and large circumstantial. These data are primarily indirect evidence of a mechanism and do not provide direct evidence for decreased estradiol levels. The fact that LH levels are above normal in the face of low E2 levels could indicate a decrease in number of LH receptors on the ovary or a decrease in the affinity of LH for its receptor. There could also be abnormal steroidogenesis in the ovary. Although serum levels were normal there could be a deficiency in some other androgen or steroid precursor or perhaps a decreased aromatase activity. However, as illustrated before, low E2 levels could themselves cause the high amplitude LH pulses through disruption of the normal feedback mechanisms.

The defect might also be at the pituitary level. Boyden (1984) found a progressive decrease in gonadotropin responsiveness to exogenous GnRH as women increased the number of miles of training in preparation for a marathon. Here too, there could be a decrease in the number of GnRH receptors or in the affinity of GnRH for its receptors. There might also be a decrease in the LH storage pool since it is estradiol that stimulates synthesis in this pool.

At the hypothalamus there are a number of factors which might disrupt normal GnRH release. Although no differences in serum B-endorphin were seen after a one hour run, this may

not reflect what is happening centrally. Daily exercise-induced rises in central B-endorphin could inhibit GnRH release. Also, exercise-induced rises in norepinephrine have been documented in a number of studies (Strauss, 1984). If norepinephrine in fact stimulates GnRH release (Yen, 1980; Rosner, 1976) this could account for the higher mean LH levels seen in runners. It remains to be seen whether other factors implicated in the control of GnRH release such as dopamine and prostaglandin E2 are effected by exercise. One study has shown an increase in catecholestrogens after an intense period of training (Russell, 1984a) but no study to date has examined the effect of acute exercise on this compound.

In summary, this study found that trained long distance runners have higher mean LH levels and higher LH pulse amplitudes when compared to a group of sedentary, age and weight-matched controls. Runners also had significantly lower follicular phase estradiol levels than controls and subnormal luteal phase progesterones. Maximal exercise evoked a dramatic increase in serum B-endorphin levels in both runners and non-runners but no changes in steroid gonadotropin levels or in cortisol. In addition, a one hour training run caused a significant rise in serum estradiol and a large increase in serum testosterone.

Much valuable information has been derived from current research on exercise-associated amenorrhea. However, studies on the amenorrheic athlete have not succeded in uncovering

the mechanism by which exercise disrupts reproductive hormone levels and normal menstrual cyclicity. In this study, highly trained eumenorrheic runners were found to have differences in gonadotropins and steroid hormones both at rest and in response to exercise compared to sedentary controls. These differences may provide insight into the process by which menstrual irregularities develop in endurance athletes.

VII. APPENDICIES

1. Volunteer agreement
2. Physical activity questionnaire
3. Menstrual history
4. Bruce protocol
5. Contraindications for continuing a graded exercise test
6. Skinfold measurement sites
7. Equations for body fat determination by skinfolds
8. Reagents for Radioiodination
9. Reagents for Radioimmunoassay
10. Formula for the correction of post exercise blood samples for changes in hematocrit
11. Chest lead placement for monitoring during the graded exercise test
12. Modified limb lead placement for monitoring during the graded exercise test

VOLUNTEER AGREEMENT

UNIFORMED SERVICES UNIVERSITY OF THE
HEALTH SCIENCES EXERCISE PHYSIOLOGY LABORATORY
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814

The Role of Exercise-Induced B-Endorphins on
Pulsatile Gonadotropin Release

You are invited to participate in a research project designed to study the effect of endurance exercise on blood levels of specific reproductive hormones and B-endorphin. B-endorphin is a substance produced by the body which may be involved in regulating the menstrual cycle as well as modulating one's response to pain. This study involves three or four separate visits to the laboratory. Visits two, three, and four will be at one month intervals.

Before participating in any of the procedures, you will be asked to fill out a complete medical history and several health questionnaires, and to undergo a general physical exam. If the findings of the physical examination are normal and indicate no apparent risk, and the medical history reveals no evidence of a condition which precludes your safety, you will be eligible to participate in the experimental protocols that have been marked below, to the left of the description.

I Experimental Protocols - Applicable Protocols Indicated in Boxes

A. Visit 1

Your first visit will be of approximately three hours duration and will include one or more of the following procedures.

☐ 1. Maximal Exercise Test

You will be asked to walk and/or jog up an incline on a treadmill for 3 to 21 minutes, depending on your level of physical fitness. The intensity of the exercise will be increased progressively until you have attained your maximal exercise capacity. During the exercise, your exercise capacity will be determined by measuring the amount and composition of the air which you breathe out of your lungs through a mouthpiece apparatus.

There are some risks associated with maximal exercise: there is some discomfort associated with strenuous exercise, there is a possibility that previously undetected heart disease may be uncovered, and there is a chance that you might experience chest pain, heart attack and/or sudden death during the procedure. A physician will be present during and after the maximal exercise test, and emergency equipment is present at all times in the lab.

☐ 2. Body Composition and Residual Lung Volume

Your weight and height will be determined by standard procedures, and your percent body fat will be determined by two methods, skinfold thickness and underwater weighing. Skinfold thickness will be

measured at seven different sites and the sum of the measures will be used in an equation to estimate body fat.

Your weight underwater will be taken as you lower yourself briefly into a tub of water, approximately 5 feet deep, while sitting on a swing. This will enable us to determine what portion of your weight is due to fat and what portion is due to lean tissue, such as muscle or bone. During this procedure you will be asked to blow out the air in your lungs while underwater and then to remain submerged for approximately 5 seconds. You will be asked to repeat this procedure up to 8 times and will be allowed to rest in between weighings.

A measurement of residual volume is necessary to calculate percent body fat. Your residual lung volume is the volume of air left in your lungs after you expel as much air as possible. Residual lung volume is measured by means of a spirometer with helium dilution. This involves breathing into a machine containing a small amount of a harmless gas, helium.

There is a chance that you may feel dizzy as a result of expelling the air from your lungs for determination of residual lung volume or percent body fat. There is also a chance that you may swallow water or get water in your mouth or nose during underwater weighing. Every precaution will be taken to ensure your safety. The other procedures are not associated with any discomforts, and pose no specific immediate or potential future risks to you.

☐ 3. Blood Drawing

One 20 ml sample (approximately 4 teaspoons) of blood will be taken by arm venipuncture. The sample will be taken prior to treadmill exercise.

Samples will be taken by a physician or a trained technician. You may experience some discomfort with the blood drawing, and possibly some bruising and/or pain at the site of blood drawing.

B. Visit 2

Subjects who are trained runners will be asked to return to the laboratory for the following procedures which will last about 2 hours.

☐ 1. Extended Submaximal Exercise Test

You will be asked to undergo a submaximal exercise test, that is, a test which ends before complete exhaustion is reached. This will involve a one hour treadmill run at a pace approximately equal to a normal training run (75% of your maximum oxygen consumption measured on day 1).

Again there are some risks associated with exercise: you may experience some discomfort during submaximal exercise, and there is the possibility that undetected heart disease may be uncovered. Also, you might experience chest pain and/or a heart attack and/or sudden death. It is highly unlikely that this will occur, but the possibility does exist. Emergency equipment and a physician will be present during this test.

☐ 2. Blood Drawing
Before and after this run a 20 ml sample (approximately 4 teaspoons) of blood will be drawn in order to measure specific hormones and B-endorphin levels. Samples will be drawn by arm venipuncture.

Blood will be drawn by a physician or a trained technician. You may experience some discomfort with drawing of the blood, and possibly some bruising and/or pain at the site of blood drawing.

C. Visit 3
This visit will last about 6.5 hours.

☐ 1. Six Hour Blood Drawing
An indwelling catheter will be inserted into each arm for the duration of your visit. One catheter will be used for blood sampling. A 10 ml (approximately 2 teaspoons) blood sample will be taken 30 minutes after catheter insertion and following this a 2 ml (less than ½ teaspoon) blood sample will be drawn every 15 minutes for six hours. The total amount of blood taken on this day will be 58 ml (about 4 tablespoons).

The catheter will be inserted by a physician and a physician will be available throughout the blood collection. You may experience some discomfort with catheter insertion, and possibly some brusing and/or pain at the site of insertion. A small risk of infection exists in catheter insertion. Sterile techniques will be employed throughout this procedure.

D. Visit 4
This visit will last about 6.5 hours.

☐ 1. Six Hour Blood Drawing
Some subjects will be asked to repeat the blood drawing described under the heading "Visit 3." The procedures and risks will be identical to those described above.

II Potential Benefits to You

After the study you will be given your maximum oxygen consumption and your percentage of body fat. Both of these may be useful in assessing your level of fitness and in planning an exercise program. You will also receive a summary of the findings of the study. As a result of your participation we may gain information on the cause and possible modes of treatment of exercise related menstrual problems.

III Protection of Your Privacy

Your name and results will remain confidential. USUHS will not use your name in the data processing or publication of the data, nor will we release your personal data to anyone without your consent.

IV Recourse in the Event of Injury

The Department of Defense (DoD) will provide medical care for DoD eligible members (active duty personnel, dependents, and retired

military personnel) for physical injury or illness incurred as a result of participation in this research. Such care may not be available to other research participants. Compensation may be available through judicial avenues to non-active duty research participants if they are injured through negligence on the part of the researchers involved in the study.

If at any time you believe that you have suffered an injury or illness as a result of participating in these physical fitness related studies, you should contact the Office of Grants Management at the Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799, at telephone number 202/295-3303. The office can review the matter with you, provide information about your rights as a subject, and may be able to identify resources available to you. Information about judicial avenues of compensation is available from the University's General Counsel at 202/295-3028.

V Withdrawl from the Study

You may withdraw your consent to participate in this study at any time without prejudice to future contacts with the Uniformed Services University of the Health Sciences.

IF YOU HAVE ANY QUESTIONS, PLEASE ASK THEM

I have read the explanation of these studies. The testing procedures have been reviewed and all of my questions have been answered.

If I have any additional questions, I understand that I may contact Janet Yu-Yahiro or Dr. Eric Schoomaker at 202/295-3623 or 202/295-3511 at USUHS. They have agreed to discuss any questions I may have about the study or the results of my test. You will be provided a copy of this consent form.

I AGREE TO PARTICIPATE IN THE FOLLOWING PROCEDURES:

1. Maximal Exercise Test ☐
2. Body Composition and Presidual Lung Volume ☐
3. Blood Drawing: Visit 1 ☐ Visit 2 ☐ Visit 3 ☐ Visit 4 ☐
4. Submaximal Exercise Test ☐

Printed Name_____

Signature_____

Date_____

Witness_____

PHYSICAL ACTIVITY QUESTIONNAIRE

Name_____ Age____ Date_____

I. General Activities

1. Besides running, do you do any other types of exercises on a regular basis? How many hours per week for each activity? (Check as many as applicable.)

	Activity	Hours Per Week
a.	Ride a bicycle	
b.	Do aerobics	
c.	Lift weights	
d.	Swim	
e.	Walk for exercise	
f.	Others (please specify)	

2. How many hours a week do you devote to the following activities:

		0 hours	1-10 hours	10-19 hours	20-29 hours	30-39 hours	40+ hours
a.	Exercise						
b.	Job						
c.	Homemaking						
d.	Volunteer activities						
e.	Social Activities (other than sports events)						
f.	Others (if more than 7 hours a week)						

3. How much of your waking/non-exercising time is spent on your feet?

   a. all of it
   b. 3/4
   c. 1/2
   d. 1/4
   e. less than 1/4
   f. almost none

II. Running History

1. What is the average number of miles you run per week?_____
2. On the average, how many days per week do you run?_____
3. On the days that you run, how many workouts do you do?_____
4. What is your average time per mile during distance training (not including speed workouts)?_____
5. Do you do interval training (speed workouts)?_____ If yes, how many times per week?_____ Describe them.

6. Within the last three years, what is your best time run for the following distances? Complete those items applicable giving your time and the year in which you achieved this time.

	Time	Year
a. 1 mile	_____	_____
b. 3 miles	_____	_____
c. 5 miles	_____	_____
d. 10 K	_____	_____
e. 10 miles	_____	_____
f. 20 K	_____	_____
g. Marathon (26 mi, 385 yds)	_____	_____
h. Ultra marathon (specify distance[s])	_____	_____

7. Approximately how many days during the past year did you run?_____
8. How many days during the past year were you unable to run because of illness or injury?_____
9. In the last three years, what kind of illnesses or injuries have you had that prevented or decreased the intensity of your workouts?_____
10. At what age did you begin long distance running?_____

MENSTRUAL HISTORY

Name_____ Age_____ Date_____

1. At what age did you first begin having menstrual periods? Put 0 if you never had a period._____
2. Have your menstrual cycles been regular, that is occurring approximately every 28 days since they first began?_____
3. Are your menstrual periods ever over two days early or two days late?_____ 5 days early or 5 days late?_____
4. What is the average length in days of your menstrual periods?_____ Between periods?_____
5. Have you ever skipped more than one period?_____ If so, how long were you without a menstrual period?_____
6. How many menstrual periods have you had within the past year?_____
7. Would you classify your average menstrual flow as light, moderate or heavy?_____
8. Do you experience premenstrual cramping or swelling?_____
9. Please give the date, length, and flow (light, moderate, heavy) of your last 2 menstrual periods_____

   _____
10. Do you now or have you previously used oral contraceptives?_____ If so, what type(s)?_____ How long did you use each type?_____
11. What is your weight now?_____ One year ago?_____ Five years ago?_____
12. Are you now or have you within the last year been dieting to lose weight?_____
13. Are you currently taking any medications?_____ If so, what medications?_____

The Following Questions are Optional

14. Have you recently been under any unusual or excessive psychological stress (i.e., divorce, child leaving home, losing your job, death in the family, etc.)?_____
15. Have you ever been pregnant?_____ If so, please give the month and year of prior deliveries_____
16. Have you had any miscarriages?_____ Abortions?_____

BRUCE PROTOCOL

I. Preliminary Data Subject_____

Resting Heart Rate_____ Resting Blood Pressure____/____

Age_____ Test Administrator(s)_____

II. Graded Exercise Test

Stage	Duration (minutes)	Time (minutes)	Speed (mph & m/min)		Grade (%)	Heart Rate	Blood Pressure
1	3	1-3	1.7	45.6	10	____	____/____
2	"	4-6	2.5	67.0	12	____	____/____
3	"	7-9	3.4	91.2	14	____	____/____
4	"	10-12	4.2	112.6	16	____	____/____
5	"	13-15	5.0	134.1	18	____	____/____
6	"	16-18	5.5	147.5	20	____	____/____
7	"	19-21	6.0	160.9	22	____	____/____
Recovery		2 minutes	sitting			____	____/____
		4 minutes	"			____	____/____
		6 minutes	"			____	____/____
		8 minutes	"			____	____/____

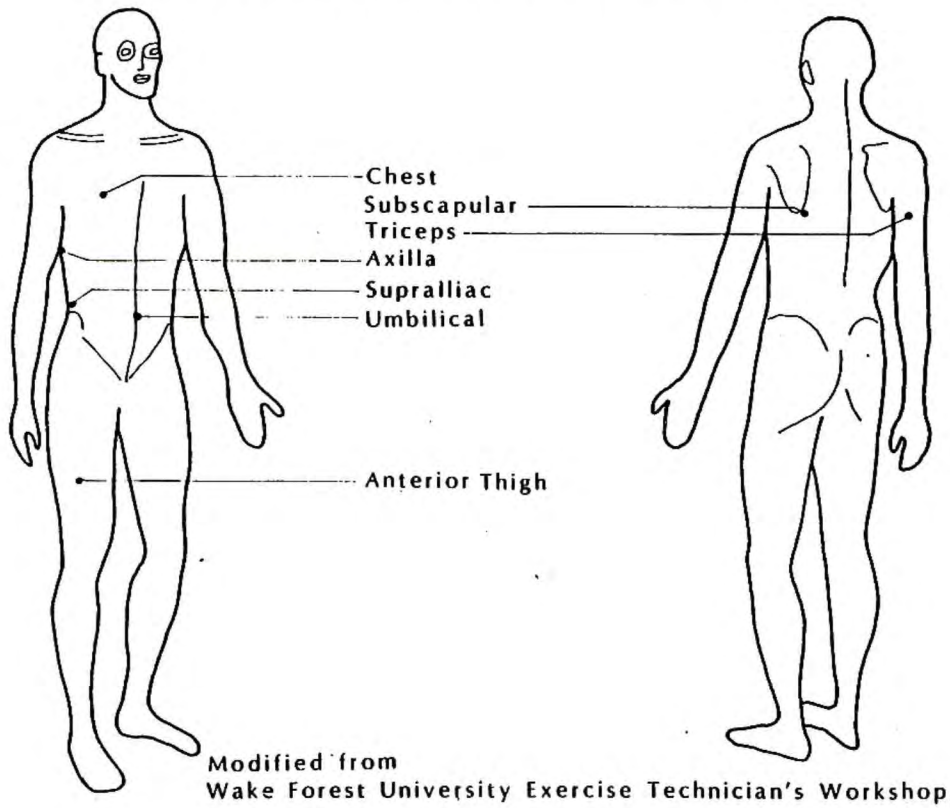
Comments:

CONTRAINDICATIONS FOR CONTINUING A GRADED EXERCISE*

1. Symptom of significant exertional intolerance
   a. Dizziness on near syncope
   b. Angina
   c. Unusual or intolerable fatigue
   d. Intolerable claudication or pain
2. Signs of intolerance
   a. Staggering or unsteadiness
   b. Mental confusion
   c. Facial expression signifying disorders
   d. Cyanosis or pallor (facial or elsewhere)
   e. Rapid distressful breathing
   f. Nausea or vomiting
   g. Definite fall in systolic blood pressure with increasing work load
3. Electrocardiographic changes
   a. S-T segment displacement of 0.2 mV below the baseline
   b. Supraventricular or ventricular dysrhythmias and ectopic ventricular activity occurring before the end of a T-wave (R on T phenomenon). It is recommended that a test be terminated in the presence of three or more successive ectopic ventricular complexes or with a significant increase in their occurrence (about 10 per minute depending on clinical judgment).
   c. Major left ventricular conduction disturbances
4. Abnormal blood pressure response
   a. Systolic pressure. During the graded exercise test, if the systolic blood pressure shows a marked decrease while the work load is further increased or if it remains constant at high work loads, the test should be terminated. There is a difficulty in setting a precise level of blood pressure change which would warrant termination of the test; however, an irreversable drop in systolic pressure will usually be the result of a significant cardiac dysfunction.
   b. Diastolic pressure. Diastolic pressure during a graded exercise test is also useful in providing information about when to stop a test. A rise in diastolic blood pressure above 100 mm Hg or a 20 mm Hg rise from the resting value is indicative of an inadequate adjustment of the vascular system to the increased activity and therefore the test in most instances would be terminated. It is advised that if diastolic pressures are to be used, phase 4 sound be adopted as the diastolic end point.

*American College of Sports Medicine (1975)

SITES OF SKINFOLD MEASUREMENTS



Modified from
Wake Forest University Exercise Technician's Workshop, 1978

EQUATIONS FOR BODY FAT DETERMINATION BY SKINFOLDS

1. FEMALES:
   Density(D)=1.10938 - 0.0008267(Sum of 3 Skinfolds**) +
   0.0000016(Sum of 3 Skinfolds)2 - 0.0002574(Age in Years)

   *(Chest + Thigh + Abdomen + Subscapula + Triceps + Suprailliac + Axilla)

   **(Chest + Abdomen + Thigh)

   Pollock, et al., British Journal of Nutrition, 40: 497-507, 1978.

2. Percent Fat=(4.95/Density) - 4.5 x 100

   Siri, W. E. Body composition from fluid spaces and density. In: Techniques for measuring body composition (Brozek and Henschel, eds.) Washington, D.C.: National Academy of Science, 1961.

REAGENTS FOR RADIOIODINATION

Chloramine-T 2ug/ul

10 mg chloramine-T, add to 5 ml vol. flask. Dilute to mark with 0.05 M $NaPO_4$, pH 7.5 (make fresh day of use).

0.5 M Sodium Phosphate

Mix 0.5 M Na_2HPO_4 with 0.5 NaH_2PO_4 to give pH of 7.5 (store frozen in 1 ml aliquots).

Na Metabisulfite ($Na_2S_2O_5$) 2.4 ug/ul

25 mg $Na_2S_2O_5$ - add to 10 ml vol. flask. Dilute to mark with 0.05 M PO_4, pH 7.6 (for disc electrophoresis, add 0.16 gram sucrose to 1 ml of $Na_2S_2O_5$) (make fresh day of use).

Transfer Solution

100.0 mg KI (add to 10 ml vol. flask)

1.0 mg bromphenol blue

Dilute to mark with 16% sucrose (1.6 gm/10 ml H_2O)

(Store frozen in 1 ml aliquots)

EQUIPMENT NEEDED FOR IODINATION

1. Disposable columns of Bio-Gel P 60 or columns of polyacrylamide for disc electrophoresis, etc.
2. Beaker with 0.05 M sodium phosphate, pH 7.5 and disposable pipette (for elution of column).
3. One ml serum bottles with hormone (2.5 ug/2.5 ul H_2O).
4. Rack with the following (each lying beside a tube):

   a. 25 ul disposable pipette in holder
   b. 10.0 ul Hamilton syringe (dry and cleaned)
   c. Tuberculin syringe with 1½", 26 gauge disposable needle with 15 ul chloramine T
   d. " " " " 50 ul $Na_2S_2O_5$
   e. " " " " 100 ul transfer solution
   f. " " " " 70 ul rinse solution
   g. Extra stopper
   h. 2 extra tubes

5. Tube of 0.5 M PO_4, pH 7.5
6. Rack of at least 10 tubes 1 ml H_2O in #1, 2, 7, 8, 9, 10 and 1 ml 5% E.W.-PBS in #3, 4, 5, 6.
7. Stop watch.

REAGENTS FOR RADIOIMMUNOASSAY

PBS (0.14 M NaCl, 0.01 M $NaPO_4$, pH 7.0, with 1:10,000 merthiolate)

1. Weigh 143 gms. NaCl in 1000 ml beaker. Dissolve in approximately 500 ml distilled water.
2. Add 1.75 gms. merthiolate.
3. Add 120 ml. 0.5 M NaH_2PO_4
4. Add 240 ml. 0.5 M $Na_2 HPO_4$
5. Dissolve completely and transfer to large storage bottle.
6. Dilute to 17.5 liters with distilled water (rinse stock beaker several times). Check pH (should be 7.0). Store at 0-4 C.

0.5 M Sodium Phosphate (monobasic)

1. Weigh 69.005 gm. NaH_2PO_4 H_2O in a beaker.
2. Transfer to a 1 liter volumetric flask and add approximately 500 ml distilled water. Rinse beaker several times.
3. Place on magnetic stirrer until completely dissolved.
4. Remove magnet with use of second magnet on outside and pulling up flask.
5. Bring to 1000 ml with distilled water.
6. Store in 1 liter bottle at 0-4 C.

0.5 M Sodium Phosphate (Dibasic)

1. Weigh 70.98 gm of Na_2HPO_4 in a beaker.
2. Transfer to a one liter vol. flask and add approximately 500 ml luke warm distilled water. Rinse beaker several times.
3. Place on magnetic stirrer until dissolved completely (a solid cake will form which takes some time to dissolve).
4. Remove magnet with second magnet.
5. When solution is at room temperature, bring to 1000 ml with distilled water.
6. Store in 1000 ml bottle at 0-4 C.

PBS-1% E.W.

1. Place 990 ml PBS in beaker over magnetic stirrer.
2. Add 10 gms powdered egg white (Sigma Chemical Co.)
3. Add 10 ml 1:100 merthiolate in PBS (1 gm/100 ml PBS, store at 0-4 C in brown reagent bottle).
4. Stir until dissolved.

PBS-1% E.W. (continued)

5. Store and freeze in regular freezer in 100 ml aliquots in Parafilm-covered milk dilution bottles.

0.05 M EDTA-PBS, pH 7.6

1. Weigh 18.6125 gm disodium EDTA (ethylenedinitrilo tetraacetic acid, disodium salt) in a beaker.
2. Add approximately 800 ml PBS. Warm and stir until dissolved. (This is difficult to dissolve in a cold solution).
3. Bring pH to 7.6 by careful addition of 5 N NaOH while stirring.
4. Transfer to 1 liter volumetric flask. When at room temperature, dilute to mark with distilled water.
6. Store at 0-4 C.

B-endorphin Assay Buffer

1. 0.05M PO_4^{-2} buffer
2. 0.05% Bovine Serum Albumin
3. 0.02% NaN_3
4. 5 mg% Bacitracin
5. 0.1N Glacial Acetic Acid

Dextran and Charcoal Suspension (1 liter)

1. Add 5 gm of charcoal to storage bottle containing stirring bar.
2. Weigh 0.5 gm T-70 Dextran, add to 1 liter 0.1% PBS - Gelatin while stirring. Dissolve completely.
3. Add to storage bottle containing charcoal. Mix thoroughly.
4. Store at 4°.

Formula for the Correction of Post Exercise Values for Changes in Hematocrit

$$CO_E = \frac{Hct_2(100-Hct_1) \times [CO_1]}{Hct_1(100 - Hct_2)}$$

Where:

CO_E = post-exercise concentration of (X) expected due to change in hematocrit alone

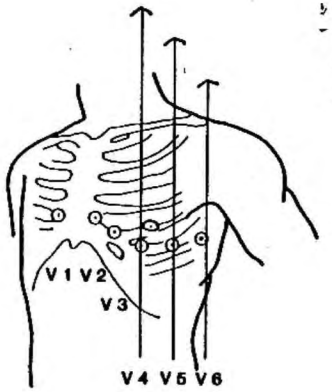
CO_1 = pre-exercise concentration of (X)

Hct_1 = pre-exercise hematocrit

Hct_2 = post-exercise hematocrit

Van Beaumont (1973)

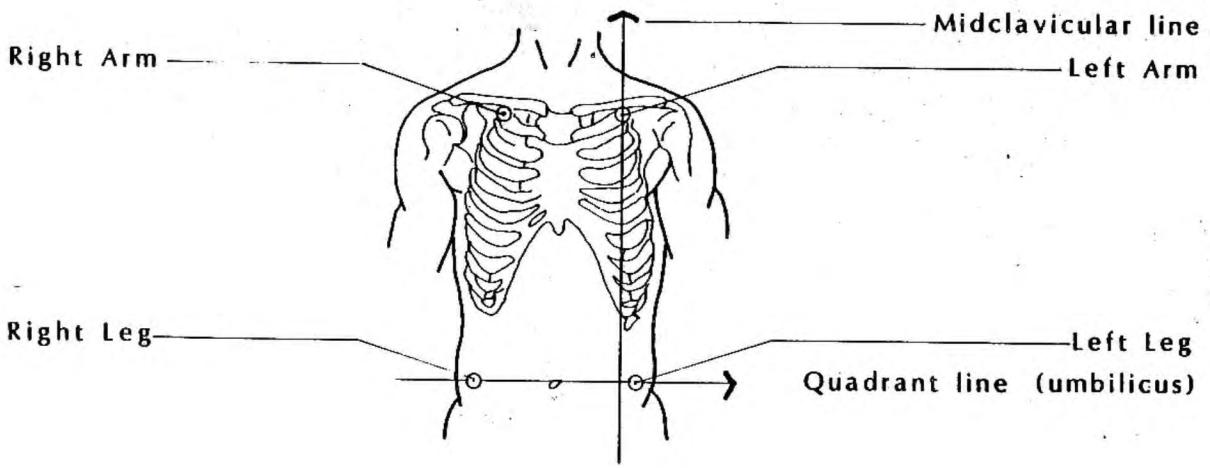
CHEST LEAD PLACEMENT FOR MONITORING DURING THE GRADED EXERCISE TEST



Midclavicular Line
Anterior Axillary Line
Midaxillary Line

Modified from "Guidelines for Graded Exercise Testing" in the Wake Forest University Exercise Technician Workshop Notebook, 1978

MODIFIED LIMB LEAD PLACEMENT FOR MONITORING DURING THE GRADED EXERCISE TEST



Modified from "Guidelines for Graded Exercise Testing" in the
Wake Forest University Exercise Technician Workshop Notebook, 1978

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